ADDIS ABABA UNIVERSITY SCHOOL OF GRADUATE STUDIES

EVALUATION OF THE DIURETIC AND ANALGESIC ACTIVITIES OF THE RHIZOMES OF *RUMEX ABYSSINICUS* JACQ IN MICE

BY: TESHALE MEKONNEN SEMRE

October, 2008

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By

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfilment for Master Degree of Pharmacy in Pharmacology.

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Acknowledgements

Above all, praise to 'Almighty God' for giving me the strength, patience and courage through out my career.

I would like to express my deepest gratitude to my supervisor Dr. Ephrem Engidawork for his continuous follow-up advices and encouragements. He genuinely and kindly supported me with everything that I was in need of. Thank you Dr. Ephrem, I simply say I learned a lot from you in the whole period of the program.

I am also happy for the genuine assistances of other members of the department.

I am also indebted to EHNRI for their kind assistance of materials and facilities to perform all my experiments. I would like to use this opportunity to appreciate the willingness Ato Kelbessa Urga (also my co-supervisor) showed to support me in everything I was demanding to. I am also grateful to the staff of EHNRI (Dani, Hewi, Dere, Wale Mesay, Teshe and others) who were consistently helping me in the process. I am also thankful to Pawlos who generously made me use the facilities and chemicals to measure the urinary electrolytes and I appreciate the initiation he had to do so.

I would like to express my deepest gratitude to Ephi, who made me use his office, and other facilities in the writing up of this thesis, of course, that is what only a genuine friend does. It is also my pleasure to thank my friend, Sol, who assisted me in some part of the experiments directly. I am also grateful to John, Chu, Mekit, Asne, Kebe, Tesfish and other friends who contributed in one way or another for the completeness this important work in my life.

I am so happy to have this opportunity for expressing my innermost appreciation to all my **FRIENDS** who were always with me for my troubles throughout. I am really lucky to have such friends!

Finally, special thanks goes to my mom and other family. I am really grateful to confess that all my achievements here are just because of my mom. Thanks mom!

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HR: Hager's Reagent

IASP: International Association for the Study of Pain

ISE: Ion Selective Electrode

LRF: Lateral reticular formation

M10: Morphine (10 mg/kg)

MR: Mayer's Reagent

NHE3: Na^+/H^+ exchanger

NKCC2: $Na^+/K^+/2Cl^-$ cotransporter

NMDA: N-Methyl-D-Aspartate

NOS: Nitric oxide synthetase

NSAIDs: Nonsteroidal anti-inflammatory drugs

PAG: Periaqueductal Gray

PCT: Proximal convoluted tuble

PG: Prostaglandin

PGD_2: Prostaglandin D_2

PGE_2: Prostaglandin E_2

$PGF_{2\alpha}$: Prostaglandin $F_{2\alpha}$

RA500: Aqueous extract of *Rumex abyssinicus Jacq* (500 mg/kg)

RA750: Aqueous extract of *Rumex abyssinicus Jacq* (750 mg/kg)

RA1000: Aqueous extract of *Rumex abyssinicus Jacq* (1000 mg/kg)

RM250: 80% methanolic extract of *Rumex abyssinicus Jacq* (250 mg/kg)

RM500: 80% methanolic extract of *Rumex abyssinicus Jacq* (500 mg/kg)

RM750: 80% methanolic extract of *Rumex abyssinicus Jacq* (750 mg/kg)

TGF: Tubuloglomerular feedback

TW80: Tween 80 (4%)

Abstract

Rumex abyssinicus Jacq (Polygonaceae) is a widely spread medicinal plant used traditionally for treatment of several ailments, including hypertension, inflammatory and painful conditions. The present study aimed to examine the diuretic and analgesic activities of aqueous and 80% methanol extracts of the rhizomes of the plant at different doses in mice.

To this effect, negative controls were orally treated with distilled water (DW) or Tween 80(4%) (TW80), solvents used for reconstitution of the extracts. Positive controls were treated with furosemide (10 mg/kg) (Fr10) for diuretic test or aspirin (100 mg/kg) (ASA100) and morphine (10 mg/kg) (M10) for acetic acid-induced writhing and hot-plate analgesic studies, respectively. For the diuretic study, treatment groups received an oral dose of 500 mg/kg (RA500), 750 mg/kg (RA750) or 1000 mg/kg (RA1000) of the aqueous extract or 250 mg/kg (RM250), 500 mg/kg (RM500) or 750 mg/kg (RM750) of 80% methanolic extract. Urine volume was then measured at different time (1, 2, 3, 4, and 5 h) and the urinary Na^{+}, K^{+} and Cl^{-} also measured at 5 h. For both analgesic tests, 250 mg/kg (RM250), 500 mg/kg (RM500) or 1000 mg/kg (RM1000) of 80% methanolic extract doses were used. Whereas the number of writhes was counted for 20 min just 5 min after intraperitonial injection of 0.6% acetic acid (0.15 mL/10g) for the writhing test, the reaction time of each mouse was evaluated at 30, 45, 60, and 90 min after treatment for the hot-plate test. For the acute toxicity study, 5000 mg/kg of aqueous or 80% methanolic extract was administered orally and observed for the following 15 days.

Both extracts displayed a clear dose-dependent diuretic and analgesic effect as compared to controls. RA1000 and RM750 were able to increase diuresis significantly *($P<0.001$)* compared to controls. Both extracts also increased urinary excretion of electrolytes, with RA1000 producing an increase by 56.9, 54.9 and 93.6% *($P<0.001$)* and RM750 increasing by 78.9, 99.5 and 76.3% ($P<0.001$) of Na^{+}, K^{+} and Cl^{-}, respectively, compared to controls. The diuretic and saluretic effects of the extracts were also found to be qualitatively similar to that of Fr10. RM1000 was noted to reduce the number of acetic acid-induced writhing by 67.6% (*$P<0.001$*) compared to controls and this effect was comparable to that of ASA100 (72.36%, *$P<0.001$*). RM1000 also conferred more than 70% protection against thermally-induced pain stimuli after 45 min of treatment compared to controls. The extracts did not show overt toxicity in mice in the study period.

In conclusion, the extracts had been shown to exert diuretic and analgesic activities comparable to that of the standard drugs and to be relatively safe. Hence, they could be considered as a potential alternative diuretic and analgesic agent. Moreover, the study lends support to the ethnobotenical use of the plant as diuretic and analgesic in different parts of Ethiopia.

Keywords: *Rumex abyssinicus* Jacq, Diuretic effect, Saluretic effect, Analgesic activity, Acetic acid-induced writhing test, Hot-plate test.

1. INTRODUCTION

1.1. Diuresis and Diuretics

A diuretic is any drug or herb that elevates the rate of urine flow and sodium excretion. Technically, a "diuretic" is an agent that increases urine volume, while a "natriuretic" causes an increase in renal sodium excretion. Because natriuretics almost always also increase water excretion, they are usually called diuretics *(Harlan, 2007)*.

Diuretics not only alter the excretion of Na^+ but also may modify renal handling of other cations (*e.g.*, K^+, H^+, Ca^{2+}, and Mg^{2+}), anions (*e.g.*, Cl^-, HCO_3^-, and $H_2PO_4^-$) and uric acid *(Jackson, 2006; Harlan, 2007)*.

Looking the historical background of diuretics, they have been available since the 16th century for the treatment of edema. Mercurous chloride was known to be diuretic by Paracelsus (1493-1543). In 1930, Swartz discovered that the antimicrobial sulfonamide could be used to treat edema in patients with heart failure due to an increase in renal excretion of Na^+ *(Blumental et al., 2007)*.

Although various agents that increase urine volume have been described since antiquity, it was not until 1957 that a practical and powerful diuretic agent (chlorothiazide) became available for widespread use *(Harlan, 2007)*.

1.1.1. Renal Anatomy and Physiology

To understand the action of diuretics, it is first necessary to review how the kidney filters fluid and forms urine. As blood flows through the kidney, it passes into glomerular capillaries located within the cortex. These glomerular capillaries are highly permeable to water and electrolytes. Glomerular capillary hydrostatic pressure drives (filters) water and electrolytes into Bowman's space and into the proximal convoluting tubule (PCT) *(Rang et al., 2003; Jackson, 2006; Harlan, 2007)*. About 20% of the plasma that enters the glomerular capillaries is filtered (termed filtration fraction) *(Miriam, 1998)*. The PCT, which lies within the cortex, is the site of sodium, water and bicarbonate transport from the filtrate, across the tubule wall, and into the interstitium of the cortex. About 65-70% of the filtered sodium is removed from the tubular fluid found within the PCT. This sodium is reabsorbed isosmotically, meaning that every molecule of sodium that is reabsorbed is accompanied by a molecule of water *(Richard, 2007)*.

As the tubule dives into the medulla, or middle zone of the kidney, the tubule becomes narrower and forms a loop (Loop of Henle) that reenters the cortex as the thick ascending limb (TAL) that travels back to near the glomerulus. Because the interstitium of the medulla is very hyperosmotic and the Loop of Henle is permeable to water, water is reabsorbed from the Loop of Henle and into the medullary interstitium *(Jackson, 2006)*. This loss of water concentrates the urine within the Loop of Henle. The TAL, which is impermeable to water *(Miriam, 1998)*, has a cotransport system ($Na^+/K^+/2Cl^-$ cotransporter) that reabsorbs sodium, potassium and chloride at a ratio of 1:1:2. Approximately 25% of the sodium load of the original filtrate is reabsorbed at the TAL *(Richard, 2007)*. From the TAL, the urine flows into the distal convoluting tubule (DCT), which is another site of sodium transport (~5% via Na^+/Cl^- cotransporter) into the cortical interstitium (the DCT is also impermeable to water) *(Miriam, 1998)*.

Finally, the tubule dives back into the medulla as the collecting duct and then into the renal pelvis where it joins with other collecting ducts to exit the kidney as the ureter. The distal segment of the DCT and the upper collecting duct has a transporter that reabsorbs sodium (about 1-2% of filtered load) in exchange for potassium and hydrogen ion, which are excreted into the urine. It is important to note two things about this transporter. First, its activity is dependent on the tubular concentration of sodium, so that when sodium is high, more sodium is reabsorbed and more potassium and hydrogen ion are excreted. Second, this transporter is regulated by aldosterone, which is a mineralocorticoid hormone secreted by the adrenal cortex. Increased aldosterone stimulates the reabsorption of sodium, which also increases the loss of potassium and hydrogen ion to the urine *(Harlan, 2007)*. Water is reabsorbed in the collecting duct through special pores that are regulated by antidiuretic hormone (ADH), which is released by the posterior pituitary. ADH increases the permeability of the collecting duct to water, which leads to increased water reabsorption, a more concentrated urine and reduced urine outflow. Nearly all of the sodium originally filtered is reabsorbed by the kidney, so that less than 1% of originally filtered sodium remains in the final urine (*Miriam, 1998; Richard, 2007*).

1.1.2. Mechanisms and Sites of Actions of Diuretics

Most diuretics produce diuresis by inhibiting the reabsorption of sodium at different segments of the renal tubular system. Sometimes a combination of two diuretics is given because this can be significantly more effective than either compound alone (synergistic effect). The reason for this is that one nephron segment can compensate for altered sodium reabsorption at another nephron segment; therefore, blocking multiple nephron sites significantly enhances efficacy *(Richard, 2007)*. Tubule transport system and sites of action of common diuretics is shown in Fig. 1.1.

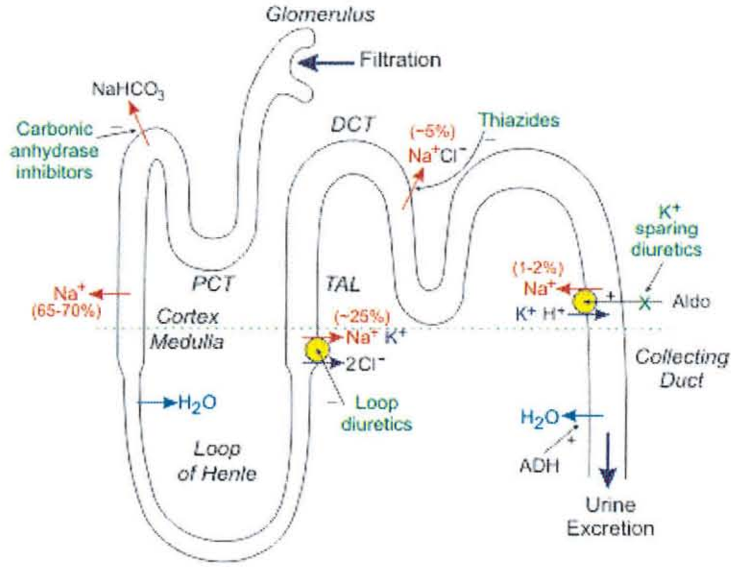

Fig.1.1. Tubule transport systems and sites of action of diuretics (Copied from *Richard, 2007*)

Loop diuretics (e.g. furosemide, bumetanide, ethacrynic acid, torsemide) inhibit the sodium-potassium-chloride co-transporter in the TAL. By acting on the TAL, which handles a significant fraction of sodium reabsorption, loop diuretics are known to be very powerful diuretics (high ceiling diuretics) *(Chennavasin, 1979; Puscas et al., 1999).*

Thiazide diuretics (e.g. bendroflumethiazide, chlorthiazide, hydrochlorthiazide, methylclothiazide, chlorthalidone, indapamide), which are the most commonly used diuretics; inhibit the sodium-chloride transporter in the distal tubule. They are sufficiently powerful to satisfy most therapeutic needs requiring a diuretic *(O'Brien and Shorten, 2001).*

Unlike loop and thiazide diuretics, some of potassium-sparing diuretics do not act directly on sodium transport. Some drugs in this class antagonize the actions of aldosterone (aldosterone receptor antagonists e.g. spironolactone, canrenone, eplerenone) at the distal segment of the distal tubule *(Harlan, 2007).* This causes more sodium (and water) to pass into the collecting duct and be excreted in the urine. They are called K^+-sparing diuretics because they do not produce hypokalemia like the loop and thiazide diuretics. The reason for this is that by inhibiting aldosterone-sensitive sodium reabsorption, less potassium and hydrogen ion are exchanged for sodium by this transporter and therefore less potassium and hydrogen are lost to the urine *(Rang et al., 2003).* Other potassium-sparing diuretics (e.g. triamterene, amiloride) directly inhibit sodium channels associated with the aldosterone-sensitive sodium pump, and therefore have similar effects on potassium and hydrogen ion as the aldosterone antagonists. Because this class of diuretics has relatively weak effects on overall sodium balance, they are often used in conjunction with thiazide or loop diuretics to help prevent hypokalemia *(Kau, 1978; Richard, 2007).*

Carbonic anhydrase inhibitors (CAIs) (e.g. acetazolamide, dichlorphenamide, methazolamide) inhibit the transport of bicarbonate out of the PCT into the interstitium by inhibiting the carbonic anhydrase (CA) enzyme, which leads to less sodium reabsorption at this site and therefore greater sodium, bicarbonate and water loss in the urine. In view of the large quantity of NaCl absorbed in this segment, a drug that specifically blocked proximal tubular absorption of NaCl would be a particularly powerful diuretic. No such drug is currently available *(O'Brien and Shorten, 2001; Harlan, 2007).* CAIs are the weakest of the diuretics and seldom used in cardiovascular disease. Their main use is in the treatment of glaucoma *(Harlan, 2007)*.

Osmotic diuretics (e.g. mannitol, urea) are relatively biologically inert molecules which are filtered by the glomerulus and not reabsorbed by the nephron. Their administration increases the osmolality of the tubular fluid, decreasing water reabsorption, decreasing Na^+ concentration of the fluid and hence its reabsorption *(O'Brien and Shorten, 2001; Harlan, 2007)*.

1.1.3. Therapeutic Uses of Diuretics

Most patients with hypertension, of which 90-95% have hypertension of unknown origin (primary or essential hypertension), are effectively treated with diuretics *(Shah et al., 2004; Richard, 2007)*. The vast majority of hypertensive patients are treated with thiazide diuretics. Potassium-sparing diuretics (e.g., spironolactone) are used in secondary hypertension caused by hyperaldosteronism, and sometimes as an adjunct to thiazide treatment in primary hypertension to prevent hypokalemia *(Richard, 2007)*. The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) study *(The ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002)* provides strong evidence that thiazide diuretics are the best initial therapy for uncomplicated hypertension, a conclusion endorsed by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure *(Chobanian et al., 2003)*.

The primary use for diuretics in heart failure is to reduce pulmonary and/or systemic congestion and edema, and associated clinical symptoms (e.g., shortness of breath - dyspnea). Long-term treatment with diuretics may also reduce the afterload on the heart by promoting systemic vasodilation, which can lead to improved ventricular ejection *(Richard, 2007)*. The available data from several small trials show that in patients with chronic heart failure, conventional diuretics appear to reduce the risk of death and worsening heart failure compared to placebo. Compared to active control e.g. Angiotensine converting enzyme (ACE) inhibitors, digoxin, diuretics appear to improve exercise capacity *(Faris et al., 2006)*.

In addition, doctors prescribe certain diuretics to prevent, treat or improve variety of conditions, such as the nephrotic syndrome *(Inoue et al., 1987)*, cirrhosis *(Ochs, 1978)*, chronic venous insufficiency & edema *(James and Shobha, 2005)*, diabetes insipidus & osteoporosis *(Gronbeck et al., 1998)*, glaucoma (*Jackson, 2006; Harlan, 2007)*, epilepsy *(Jackson, 2006)*, acute mountain sickness *(Harlan, 2007)* and as an alternate treatments in asthma *(Alexander et al., 2003)*.

1.1.4. Adverse Effects of Diuretics

Thiazide and loop diuretics may cause skin reactions and interstitial nephritis *(Brater, 1998)*. Loop diuretics may cause ototoxicity, usually in patients receiving both very high doses and other ototoxic drugs, particularly aminoglycoside antibiotics *(Sheffield and Turner, 1971)*. Ototoxicity is usually transient. Large doses of spironolactone can cause gynecomastia *(Rose et al., 1977)*.

The most serious adverse effects of diuretics are abnormalities in fluid and electrolyte homeostasis. Both loop and thiazide diuretics cause loss of potassium and magnesium in the urine, and when administered in combination, they may result in substantial depletion of these cations (*Brater, 1996)*. Other classes of diuretics are also associated with various specific adverse effects (For review see *Richard, 2007*).

1.1.5. Diuretic Tolerance

There are two forms of diuretic tolerance. Short-term tolerance, so-called braking, refers to a decrease in the response to a diuretic after the first dose has been administered. The mechanism by which short-term tolerance occurs is unclear. It may be mediated by activation of angiotensin II or the sympathetic nervous system, but neither the inhibition of ACE nor adrenergic blockade, separately or together, consistently prevents it *(Kelly, 1983)*.

With long-term administration of a loop diuretic, the solute that escapes from the loop of Henle floods more distal regions of the nephron. By unknown mechanisms, increased exposure to solute causes hypertrophy of distal nephron segments, with concomitant increases in the reabsorption of sodium *(Kaissling and Stanton, 1988)*.

Sodium that escapes from the loop of Henle is therefore reabsorbed at more distal sites, decreasing overall diuresis. The result is long-term tolerance of the loop diuretic. Thiazide diuretics block the nephron sites at which hypertrophy occurs, accounting for the synergistic response to the combination of a thiazide and a loop diuretic. This phenomenon reinforces the logic of using combinations of loop and thiazide diuretics in patients who do not have adequate responses to optimal doses of a loop diuretic *(Ellison, 1991)*.

1.1.6. Future Diuretics

All currently available diuretics perturb K^+ homeostasis. However, studies in animals have established that there are some agents which induce a brisk natriuresis without significantly increasing urinary K^+ excretion *(Kuan et al., 1993)*.

Adenosine A_1 receptor antagonists

Clinical studies with highly selective A_1-receptor antagonists FK453 *(Balakrishnan et al., 1993; van Buren et al., 1993)*, KW-3902 *(Yao et al., 1994)*, CVT-124 *(Gellai et al., 1998)* and FK838 *(Christine et al., 2003)* confirm that blockade of A_1 receptors induces natriuresis with minimal effects on K^+ excretion.

The natriuretic mechanism of this novel class of diuretics has been partially elucidated *(Takeda et al., 1993)*. Elevated intracellular cyclic AMP (cAMP) reduces basolateral Na^+-HCO_3^- symport in proximal tubular cells. Endogenous adenosine normally acts on A_1 receptors in these cells to inhibit adenylyl cyclase and reduce cAMP accumulation. Blockade of A_1 receptors removes this inhibition, permits cellular cAMP to rise, and results in reduced activity of the Na^+-HCO_3^- symporter. Because A_1 receptors are involved in tubero glomerular feedback (TGF), A_1-receptor antagonists uncouple increased distal delivery of Na^+ from activation of TGF *(Wilcox et al., 1999)*. Other mechanisms, including an effect in the collecting tubules, contribute to the natriuretic response to A_1-receptor antagonists; however, it is not known why this class of diuretics has little effect on K^+ excretion. In some patients, loop diuretics may compromise renal hemodynamics and actually reduce glomerular filtration rate (GFR), a phenomenon known as *diuretic intolerance*. Importantly, A_1-

receptor antagonists tend to improve GFR in the setting of diuretic intolerance. A_1-receptor antagonists are in clinical trials as "renal friendly" diuretics for the treatment of edema owing to heart failure *(Jackson, 2002).*

Non-peptide arginine-vasopressin antagonists: the vaptans

Scientists have developed a new class of drugs called the vaptans that could treat a large variety of conditions, including but not limited to painful menstrual periods, brain hemorrhage, certain psychotic disorders, and glaucoma *(Decaux et al., 2008).*

The vasopressin-receptor antagonists, vaptans, target the vasopressin hormonal feedback system. Vasopressin, also called ADH, is an important part of regulation in the circulatory system and is integral to the balance of water in the body *(Decaux et al., 2008).*

Subclass of vaptans, which targets these cell receptor site, encompasses several drugs including mozavaptan, lixivaptan, satavaptan, and tolvaptan, all inducing water loss without the loss of mineral salts, which often accompanies other diuretics. These drugs are used to treat hyponatraemia. Presently, conivaptan is the only vaptan approved by the food and drug administration (FDA) for treatment of this condition *(Decaux et al., 2008).*

1.1.7. Herbal Diuretics

Plant medicine was commonly used for traditional treatment of some renal diseases and a lot of plants were reported to show significant diuretic activity. Many investigators demonstrated that studies of herbal plant used in traditional medicine as diuretics were in progressive elevation in the last decades, and might be a precious tool used in human pathology treatment *(Jouad et al., 2001).*

In line with this, there are an increasing number of published articles claiming that plants or plant-derived active ingredients may function as diuretic agents. A large majority of these researches have determined the degree of clinical support for the traditional use of common or folklore medicines. Such evidence is needed in order to determine whether there is any scientific basis for their use *(Wright et al., 2007).*

There are several plant species and genera reported to posses diuretic effects. Of these, the most promising, at the present time are described below: *Foeniculum vulgare (Beaux et al., 1997; El Bardai et al., 2001; Conforti et al., 2006), Fraxinus excelsior (Casadebaig et al., 1989; Eddouks et al., 2005), Hibiscus sabdariffa (Odigie et al., 2003), Petroselinum sativum* and *Spergularia purpurea (Jouad et al., 2001b)*, and species from the genera *Cucumis* (*Cucumis melo (Singh and Sisodia, 1970)* and *C. trigonus (Naik et al., 1981)*), *Equisetum* (*Equisetum bogotense, E. uviatile, Equisetum giganteum, E. hiemale* var. and *E. myriochaetum*) *(Perez et al., 1985; Lemus et al., 1996), Lepidium* (*Lepidium latifolium* and *L. sativum*), *Phyllanthus* (*Phyllanthus amarus, P. corcovadensis (Ribeiro et al., 1988)* and *P. sellowianus (Hnatyszyn et al., 1999)*) and *Sambucus* (*Sambucus mexicana (Caceres et al., 1987)* and *S. nigra (Beaux et al.,1999)*.

1.2. Pain and Analgesics

1.2.1. Physiology of Pain

Pain is a subjective experience, hard to define exactly, even though we all know what we mean by it. Pain, while complex, seems too obvious a human experience to attempt a verbal definition but the International Association for the Study of Pain (IASP) has nevertheless, after much debate, research and deliberation reduced pain to the following definition *(Merskey, 1986)*:

"An unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life. It is unquestionably a sensation in a part of the body, but it is also unpleasant, and therefore also an emotional experience. Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this happens for psychological reasons. There is no way to distinguish their experience from that due to tissue damage, if we take this subjective report".

1.2.2. Types of Pain

There are two major types of pain, nociceptive and neuropathic *(McCaffery and Pasero, 1999; Ranney, 2001; James, 2003)*. Distinguishing between them is important because the causes and treatments are different. Ideally, the causes of both types of pain will be identified and treated, resulting in pain relief *(James, 2003)*.

Nociceptive Pain

Nociceptive pain is pain in which normal nerves transmit information to the central nervous system about trauma to tissues (nocere = to injure, Latin). Stimulation of a nociceptor, due to a chemical, thermal, or mechanical event that has the potential to damage body tissue, may cause nociceptive pain *(Jensen, 1996)*.

Nociceptive pain may be classified further in three types that have distinct organic origins and felt qualities as superficial somatic pain (or cutaneous pain), deep somatic pain and visceral pain *(McCaffery and Pasero, 1999; Ranney, 2001)*.

Nociceptive pain can be experienced as sharp, dull, or aching. There may be radiation of the pain, especially visceral pain, but it will not be in a direct nerve distribution *(McCaffery and Pasero, 1999)*.

Nociceptive pain is generally responsive to NSAIDs (nonsteroidal anti-inflammatory drugs) and opioids. Conditions associated with inflammation, bone pain, and joint diseases are particularly responsive to NSAIDs *(James, 2003)*.

Neuropathic Pain

Neuropathic pain may occur when there is either damage to or dysfunction of nerves in the peripheral or central nervous system. Faulty signals are sent to the brain and experienced as pain *(James, 2003)*.

Neuropathic pain can be either peripheral or central in origin. Examples of neuropathic pain include diabetic neuropathy, trigeminal neuralgia, postherpetic zoster pain (peripheral pains), and the thalamic pain syndrome (a central pain) *(McCaffery and Pasero, 1999).*

Neuropathic pain is relatively resistant to NSAIDs and opioids, although they may be helpful in certain cases. The other major classes of medications useful for neuropathic pain are tricyclic antidepressants, anticonvulsants, and sodium channel blockers *(James, 2003).*

1.2.3. Pain Pathways

Management of pain requires a basic understanding of the neuroanatomy through which noxious stimuli are mediated and perceived.

Pain generally starts with a physical event; a cut, burn, tear, or bump *(Catalano, 1987).* The sensation of pain usually depends on the activation of a set of neurons that includes primary afferent nociceptors, interneurons in the spinal cord, cells of the ascending tracts, thalamic neurons and neurons of the cerebral cortex. Hence, the pain system involves a set of ascending pathways that convey nociceptive information from peripheral nociceptors to higher levels of the central nervous system, as well as descending pathways that modulate the information *(Bromm and Desmedth, 1995).*

The term nociception refers to the process by which pain information is carried from the periphery via sensory receptors in the skin and in the viscera to the cerebral cortex through network of neuronal relays. Exteroceptors on the body surface and propioceptors within the body are specialized neurons that receive stimulation; mechanical (e.g. pressure), chemical, electrical, or thermal (i.e. hot-cold sensitive) *(Karoly and Jensen, 1987).*

The body is equipped with mechanical nociceptors at the periphery (so-called first-order neurons), which project to second-order neurons in the spinal cord and medulla, which then carries the sensory information (in the form of electrical impulse) to the thalamus, where it synapses with third-order neurons that transmit the impulse to the cortex *(Karoly and Jensen, 1987).*

Second-order neurons send their sensory inputs to the thalamus via two ascending pathways: the dorsal column medial-lemniscal system and the anterolateral system (includes the spinothalamic, spinoreticular, and spinotectal fibers). The former transmits impulse involving position sense, touch, and pressure. The latter pathway is involved in pain transmission *(Karoly and Jensen, 1987)*.

The spinal cord is the central concourse along which all pain messages travels to and from the brain *(Catalano, 1987)*. The cells in the substantia gelatinosa relay "fast pain" messages along the neospinothalamic and terminating in the thalamus and the cortex *(Catalano, 1987)*.

In contrast, chronic pain moves along a different and slower tract, called the paleospinothalamic tract. This "slow pain" is generally dull, aching, burning, and cramping *(Catalano, 1987)*. Slow pain follows the same path as the fast pain through the spinal cord, but once in the brain, it separates and terminates in the hypothalamus and the limbic structures. The hypothalamus is responsible for stimulating the release of stress hormones *(Catalano, 1987)*.

Just as there is an ascending pain pathway from the body to the brain, there is a descending pathway that allows the brain to modulate pain sensation. The brain uses this pathway to send chemical substances and nerve impulses back down to the cells in the spinal cord to act against the pain message sent up by the pain receptors. Hence, the primary role of the descending pathway is to send chemical messages from the brain to close the gates in the spinal cord to ascending messages *(Catalano, 1987)*.

Descending inhibitory processes are of great interest in the research arena. Hence, it has been extensively studied by scientists. For instance, descending inhibitory processes have been investigated in anesthetized animals *(Zimmerman, 1984)*. It was found that the firing of dorsal horn neurons in response to noxious skin heating can be inhibited by stimulation in the periaqueductal gray (PAG) and the lateral reticular formation (LRF) in the midbrain. In addition, inhibition of the spinal cord neurons can also be achieved by electrical stimulation in other regions of the brain, such as the raphe nuclei, the locus coeruleus, and various regions of the medullary reticular formation, as well as sites in the hypothalamus, septum, orbital cortex, and sensorimotor cortex *(Zimmerman, 1984)*.

1.2.4. Analgesic Agents

i. Traditional NSAIDs

NSAIDs are among the most widely used medications. Given their efficacy in managing fever, mild to moderate pain and, at higher doses, inflammation, such widespread use is generally appropriate. It has been estimated that 20-30% of Americans use an NSAID each year, and 1-2% use NSAIDs every day (*McGoldrick and Bailie, 1979)*.

The generally accepted mechanism of action of NSAIDs is that they attenuate prostaglandin (PG) synthesis by inhibiting cyclooxygenase (COX) enzymes *(Vane, 1971)*, although some central action has been reported *(Yaksh and Malmberg, 1993)*.

NSAID side-effects, however, are often related to COX inhibition, which greatly limits their use *(Gardner and Simkin, 2000)*. The side-effects are primarily gastrointestinal (GI), haematological and renal *(Venkat et al., 1998)*.

ii. COX-2 NSAIDs

COX-2-selective NSAIDs (eg. Celecoxib, Valdecoxib, Parecoxib, Etoricoxib, Lumaricoxib) have recently received much attention from clinicians, scientists, patients and the media. As their name suggests, these agents are selective for the COX-2 enzyme, which is thought to be responsible more for pain and inflammation, whereas the COX-1 isoform more commonly provides homeostasis in the intestines, kidneys and elsewhere *(Lipsky et al., 2000)*. As a result, COX-2 NSAIDs are less likely to cause gastric ulceration, but they may inhibit healing of previous ulcers and therefore should not be used in patients with a history of GI ulcers, particularly NSAID-induced ulcers *(Lipsky et al., 2000)*.

iii. Acetaminophen

Acetaminophen (paracetamol) has been a mainstay for pain relief and fever control since its approval for use as an analgesic in 1960 *(Ameer et al., 1977).* The experimental evidence converges on two possibilities of the mechanism of action of acetaminophen: an effect at the local site of injury (peripheral action), and an effect at some level, spinal or supraspinal, of the central nervous system (central action). The peripheral action would presumably be related to inhibitory modulation of peripheral mediators of pain. Some suggestions include inhibition of nitric oxide synthase (NOS) *(Bjorkman et al., 1994)*, reversal of the hyperalgesia induced by N-methyl-D-aspartate (NMDA) or substance P *(Hunskaar et al., 1985; Bjorkman, 1995)*, decrease of spinal prostaglandin E_2 (PGE_2) release *(Muth-Selbach et al., 1999)* or an effect on spinal cord 5-hydroxytryptamine (5-HT; serotonin) *(Pelissier et al., 1995; Pelissier et al., 1996).* The effect on NOS and 5-HT would have to be indirect, as it was found that acetaminophen does not inhibit constitutive or inducible NOS or bind to known 5-HT receptors or to 5-HT neuronal reuptake sites *(Raffa et al., 2001).*

The side-effects of therapeutic use of acetaminophen are minimal. Unlike NSAIDs, it does not significantly inhibit PGs and hence does not produce GI irritation or inhibit platelet aggregation. A serious adverse effect is hepatotoxicity, which can occur with large doses (10-15 g), when glutathione stores are depleted and a toxic metabolite of acetaminophen is allowed to accumulate *(Schueler and Harper, 1995).*

iv. Opioids

The use of opium, opiate extracts (e.g. morphine and codeine) and opiate-like substances (opioids) originated more than 1000 years ago, and morphine was synthesized almost 200 years ago. Opioids are the most powerful pain relievers, and therefore traditional opioid analgesics remain the drug of choice for the medical treatment of severe acute pain syndromes and for progressive severe chronic illnesses *(Jaffe and Martin, 1985).*

An understanding of the mechanism of action of opioids has only become possible since the identification of endogenous opioid-like peptides and receptors in the early 1970s *(Benedetti and Butler, 1990).* Three major structurally and pharmacologically distinct opiate receptor types (μ, δ and κ) and their subtypes are located throughout the body, both in the central and peripheral nervous system. A fourth member of the opioid peptide receptor family, the *nociceptin/orphanin FQ* (N/OFQ) receptor, was cloned in 1994 *(Jackson, 2006)*. Analgesia is thought to primarily involve opioid receptors in the brain and spinal cord *(Jaffe and Martin, 1985)*.

The most common side-effects seen with opioid therapy are constipation, nausea, vomiting, sedation, itching and respiratory depression. Tolerance and physical dependence may also occur when using opioids over long periods of time (more than 2-3 weeks of continuous administration) *(American Pain Society, 1990)*.

v. Tramadol

Just as acetaminophen shares some, but not all, the characteristics of NSAIDs, tramadol shares some, but not all, the characteristics of opioids. Tramadol was initially described as a traditional opioid; however tramadol has low affinity for μ-opioid receptors, more akin to the nonopioid imipramine than the opioid codeine *(Raffa et al., 1992)*.

vi. Adjuvant Analgesics

In the past few years, knowledge of the neurophysiology, biochemistry and modulation of pain transmission has expanded at an increasing rate. Recently, numerous compounds have been studied to identify novel analgesic properties, including antidepressants *(Onghena and van Houdenhove, 1992; McQuay et al., 1996)*, anticonvulsants *(Backonja et al., 1998)* and clonidine *(Joshi et al., 2000; Van Elstraete et al., 2000)*. Because current therapy for pain relief is inadequate for some patients, and chronic pain is difficult to treat, the search for new analgesic compounds or therapies will continue.

vii. Future Analgesics

The search for new analgesic compounds devoid of the side effects typical of morphine-like opioid agonists as well as of the gastrointestinal irritation and kidney damage associated with NSAIDs has attracted considerable attention in recent years

(Tijen et al., 2004). With a better understanding of the anatomy and physiology of pain, there is a significant effort in developing new drugs that interact specifically with pain pathways *(Mark, 2001)*. The current research in finding promising analgesic agents includes the following:

Prostaglandin receptor antagonists: PGs are generated by most cells in response to mechanical, thermal, or chemical injury and inflammatory insults. PGE_2 especially has attained recognition as a mediator of hyperalgesia, even though significant quantities of other PGs are released by injury or inflammation. This focus on PGE_2 may have arisen because it was found to be a more potent nociceptive agent than prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin D_2 (PGD_2), or thromboxane A_2 (TXA_2) in early studies *(Ferreira, 1972)*.

PGE_2 receptors are classified into four subtypes, EP_1, EP_2, EP_3, and EP_4. PGE_2 induces c-fos expression in osteoblastic cells through the EP_1 receptor *(Suda et al., 2000)*. Receptor antagonist (EP_1-ra), ONO-8711 (Ono Pharmaceutical, Osaka, Japan) significantly reduced hyperalgasia and allodynia on rat model. These findings suggested that EP_1 receptor antagonists may have a role in treatment of neuropathic pain *(Hiroyasu et al., 2001)*.

NMDA receptor antagonists: Dextromethorphan, ketamine, and methadone block NMDA receptors. Dextromethorphan, a widely used cough preparation, produces analgesia and is well tolerated. Ketamine, a dissociative anesthetic, produces analgesia at low doses; Norketamine, a metabolite of ketamine, is also analgesic. Both isomers of methadone block NMDA receptors but only the d-isomer binds to opioids, according to rodent studies *(Conrad, 1996)*.

Adenosine kinase inhibitors: Substantial evidence exists for a physiological role of adenosine (ADO) in the modulation of primary afferent transmission in the spinal cord *(Sawynok and Sweeney, 1989; Sawynok, 1998; Dickenson et al., 2000)*. The antinociceptive properties of ADO have been demonstrated across a wide range of animal models, including acute nociceptive tests *(Sawynok, 1999)*, models of inflammation *(Karlsten* et al., *1992; Poon and Sawynok, 1998)* and neuropathy *(Lee* *and Yaksh, 1996; von Heijne et al., 1998; Sjolund et al., 1998; Lavand'homme and Eisenach, 1999).*

ADO kinase (AK; ATP:adenosine 5′ phosphotransferase) catalyses the phosphorylation of ADO to AMP (adenosine 5′-monophosphate), and is the primary enzyme regulating ADO metabolism under physiological conditions. Thus, inhibition of AK represents an alternative strategy for taking advantage of the beneficial actions of ADO by selectively increasing local concentration of endogenous ADO in a site and event specific manner *(Rie et al., 2001).*

A study was conducted to investigate the effect of a novel and potent non-nucleoside AK inhibitor, ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl) pyrido [2,3,-d] pyrimidine), on the responses of dorsal horn neurones to selected peripheral stimuli in animal models. ABT-702 is orally effective to reduce behavioural signs of nociception in models of acute, inflammatory, and neuropathic pain *(Rie et al., 2001).*

NOS inhibitors: NOS inhibitors interfere with second messenger systems involved in sensory transmission. After NMDA activation, calcium enters the neuron, NOS catalyzes the formation of nitric oxide, and excitatory amino acid release results in pain. Disrupting this pathway offers another opportunity to produce analgesia *(Conrad, 1996).*

viii. Herbal Analgesics

Millions of dollars are spent a year in the world on pain relief. With the rise of narcotic addiction and dangerous side effects from over the counter pain relievers, many people are turning to herbal therapies. Herbs are generally safe and gentle yet effective if used correctly. Herbal analgesics are cost effective and are non addictive *(Horton, 2007).* The following are some common and effective herbal analgesics: *Cissus quadrangularis* Linn (*Pongboonrod, 1995; Panthong et al., 2007*), *Viburnum opulus* (Cramp Bark) *(Horton, 2007)*, *Salix alba* (White Willow) *(Horton, 2007)*, *Cheilanthes farinosa* (*Yonathan et al. 2006*), *Leogrus cardiaca* (Motherwort) *(Horton,* *2007), Scutellaria baicalensis* (Skullcap) *(Horton, 2007), Cannabis sativa* (Cannabis), *Angelica sinensis* (Angelica) *(Horton, 2007).*

1.3. Rumex abyssinicus Jacq

Rumex abyssinicus Jacq (Familly: Polygonaceae) is a pereneal herb, up to 3-4m tall *(Demissew et al, 2000; Aluka, 2008).* It is a widely spread medicinal plant in the highlands of tropical Africa and is a common weed of cultivated lands or disturbed grounds ranging from N.Africa to Ethiopia *(Aluka, 2008).*

The rhizomes are used to refine butter and give it a rich yellow color. They are also used medicinally and extracts are drunk to control mild diabetes. Traditional practitioners also make use of the root of *R. abyssinicus* to treat patients with hypertension, migraine, rheumatism, breast cancer, stomach distention, earache, liver diseases, hemorrhoids, typhus, rabies and scabis. Traditional practitioners in Southern Region of Ethiopia use the rhizomes as a diuretic for treatment of hypertension (*Gete etal, 2003; Blumental et al, 2007).*

Most of the reported biological effects have rarely been supported by any scientific background and rely mainly on empirical information. However, studies showed that the crude extracts of the root of *R. abyssinicus* have antimicrobial and antiinflammatory activities. Several Rumex species have antimicrobial action due to the presence of physcion and rumicin. It has been also reported that *R. abyssinicus* and other Rumex species of Polygonaceae are active against *tinea nigra* and *tinea versicoulor (Abebe et al., 2003, Gete et al., 2003).*

WHO estimated that 80% of the people of the world living in developing countries rely on medicinal plants for primary health care needs *(Farnsworth, 1998).* For instance, about 70 - 80% of the Ugandan population still rely on traditional healers for day-to-day health care *(Maud and Hannington, 2005).* Traditional herbal medicine is also of great importance for local health care in Ethiopia. The demand for traditional herbal medicine is higher than ever with increasing population pressure and a failing availability of modern medicine. The prevalence of the use of herbal drugs in self-care was found to be 12.5% in rural central Ethiopia *(Gedif and Hahn, 2003).*

Moreover, studies show that 25% of modern medicines are made from plants first used traditionally *(Olusegun, 2006)*. Currently used modern antimalarial drugs such as quinine, artmesnin derivatives, digoxin glycosides, etc are good examples for this *(Olusegun, 2006)*.

Ongoing research in the area of botanical medicine continues to identify bioactive compounds in herbs and their effectiveness in providing general wellness. However, increased interest and reports on efficacy of herbs have resulted in confusion among consumers. Moreover, the increase in use of herbal products in conjunction with prescription medications has resulted in previously unrecognized drug-herb interactions (*Dennis, 2007)*. There is, therefore, a need to intensify research into medicinal flora especially those claimed to have beneficial effects in serious disorders *(Magaji et al., 2008)*.

It has been recognized that a number of plants are used as diuretic and analgesic agents traditionally and some of them are confirmed for their claimed activities by various researches. *R. abyssinicus* is one of the plants found in Ethiopia which are widely used traditionally as diuretic and analgesic. In order to advocate the ethnobotenical uses of *R. abyssinicus*, the biological effects of the plant should be supported by scientific background. Moreover, the bioactive compounds in the herb and their effectiveness in providing wellness should be confirmed scientifically.

2. OBJECTIVE (S)

2.1. General Objective

The purpose of this study was to examine the acute diuretic and analgesic activities of the aqueous and 80% methanolic extracts of the rhizomes of *Rumex abyssinicus Jacq* at different doses in mice.

2.2. Specific Objectives

- ✓ To evaluate the effect of the aqueous and 80% methanolic extracts of the plant on urine volume.
- ✓ To evaluate the effect of the aqueous and 80% methanolic extracts of the plant on excretion of urinary electrolytes (Na^+, K^+, Cl^-).
- ✓ To estimate the electrolyte content of the aqueous extract of the plant.
- ✓ To evaluate the analgesic effect of 80% methanolic extract of the plant by using acetic acid-induced writhing and hot-plate tests.
- ✓ To study the acute toxicity of the aqueous and 80% methanolic extracts of the plant after oral administration.

3. MATERIALS AND METHODS

3.1. Experimental Animals

Adult albino mice bred in the animal house of Ethiopian Health and Nutrition Research Institute (EHNRI) and having weights ranging from 25 to 35 g were used for the experiment. The animals were housed under standard environmental conditions (25±1 ◦C, 55±5% humidity and 12 h/12 h light/dark cycle). The animals were allowed free access to tap water and standard laboratory pellet. The care and handling of mice were in accordance with the internationally accepted standard guidelines for use of animals.

3.2. Collection of the Plant Material

The rhizomes of *R. abyssinicus* were collected from a place called Menagesha, a few kilometers West from Addis Ababa, Ethiopia in December 2007. The plant was identified as *R. abyssinicus* at the Herbarium of School of Pharmacy, Addis Ababa University. A voucher specimen (Voucher No. T0001) was deposited in the Herbarium.

3.3. Extraction of the Plant Material

The rhizomes of *R. abyssinicus* were sliced to smaller pieces and dried at room temperature in the shade for more than two weeks. The dried and sliced pieces of the rhizomes were then powdered finely and extracted as follows.

3.3.1. Aqueous Extraction

10 grams of the dried powder of rhizomes of *R. abyssinicus* was boiled at 100°C in 200 ml of distilled water for 10 min, cooled to room temperature for 15 min. The decoction obtained was centrifuged, filtered, frozen at −20 °C, and then lyophilized. The dried extract was collected and weighed. The approximate yield of the dry extract was 15.2% (w/w). The dried plant extract was reconstituted with distilled water (DW) for oral administration.

3.3.2. 80% Methanol Extraction

10 grams of dried powder of rhizomes of *R. abyssinicus Jacq* was macerated with about 100 ml of 80% methanol for 24 h. After 24 h, the extract was filtered and the marc was remacerated twice using the same volume of 80% methanol to exhaustively extract the plant material. The hydroalcohol was then removed from the extract by evaporation under reduced pressure using a rota vapor (BUCHI Rotavapour R-200, Switzerland) at 40 °C. The resulting dry extract was weighed and calculated for percentage yield which was 16.23% (w/w). The dried plant extract was reconstituted with Tween 80 (4%) (TW80) for oral administration.

3.4. Phytochemical Screening

Chemical tests were carried out on the aqueous and 80% methanol extracts using standard procedures to identify the constituents as described by Trease and Evans (1989), Edeoga (2005), Gopiesh and Kanabiram (2007). DW and methanol were used for dissolving the dry aqueous and 80 % methanol extracts, respectively.

3.4.1. Test for Phenolic Compounds (Ferric Chloride Test)

The extract was diluted to 5 ml with an appropriate solvent. To this a few drops of neutral 5% ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds.

3.4.2. Test for Tannins

About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

3.4.3. Test for Saponins

The extract was diluted with an appropriate solvent and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of foam indicates the presence of saponins.

uniform water and salt load *(Benjumea et al., 2005)*. Negative controls of the aqueous and hydroalcoholic extract groups were treated orally with DW and TW80, respectively and positive controls were treated orally with furosemide (10 mg/kg) (Fr10). Treatment groups received an oral dose of 500 mg/kg (RA500), 750 mg/kg (RA750) or 1000 mg/kg (RA1000) of aqueous extract or 250 mg/kg (RM250), 500 mg/kg (RM500) or 750 mg/kg (RM750) of 80% methanol extract of the plant. Immediately after administration, the mice were individually placed in a metabolic cage. Urine was then collected and measured for a total of 5 h at 1, 2, 3, 4, and 5 h after the dose, filtered and finally conserved at -20 ^{0}C for further analyses.

The following parameters were calculated in order to compare the effects of the extracts and furosemide on urine excretion. The urinary excretion independent of the animal weight was calculated as total urinary output divided by total liquid administered (1.5 ml) (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 a given dose of a drug (Formula -2). A parameter known as diuretic activity was also calculated. To obtain diuretic activity, the diuretic action of the extract was compared to that of the standard drug in the test group (Formula – 3) *(Mukherjee, 2002)*.

$$\text{Urinary Excretion} = \frac{\text{Total urinary output}}{\text{Total liquid administered}} \times 100\% \qquad \text{(Formula-1)}$$

$$\text{Diuretic Action} = \frac{\text{Urinary excretion of trated group}}{\text{Urinary excretion of control group}} \qquad \text{(Formula-2)}$$

$$\text{Diuretic Activity} = \frac{\text{Diuretic action of test drug}}{\text{Diuretic action of standard drug}} \qquad \text{(Formula-3)}$$

3.5.3. Analytical Procedures

Sodium, potassium and chloride levels of urine and the plant extract were quantitated by Ion Selective Electrode (ISE) analysis (AVL 9181 Electrolyte Analyzer, Roche, USA). The analyzer contains software which permits $Na^+/K^+/Cl^-$ parameter configuration. A 2-point calibration was performed automatically every 4 h in ready mode and a 1-point calibration was automatically performed with every measurement.

3.5.4. Analgesic Activity

i. Acetic acid-induced writhing test

The abdominal constriction test described by *Collier et al. (1968)* was used with slight modification to measure the analgesic activity of 80% methanolic extract of the rhizomes of *R. abyssinicus*. Male or female albino mice were fasted overnight with water given *ad libitum* and then pre-treated either with oral TW80 (0. 15 mL/10 g), aspirin (100 mg/kg) (ASA100), or plant extract (250mg/kg (RM250), 500 mg/kg (RM500) or 1000 mg/kg (RM1000)). 30 minutes later, all mice were treated with intraperitoneal injection of 0.6% acetic acid (0. 15 mL/1 0 g) to cause a typical stretching response. 5 minutes after acetic acid injection, mice were kept in individual cages and writhing or stretching of each mouse was counted for a period of 20 minutes by a blinded individual. The analgesic effect was measured by calculating the mean reduction in the number of abdominal constrictions for each drug, as compared to TW80 controls. Percentage inhibition of writhing was also calculated using Formula – 4.

$$\text{Inhibition (\%)} = \frac{\text{Mean Number of writhes (control)} - \text{Mean Number of writhes (test)}}{\text{Mean Number of writhes (control)}} \times 100 \quad \text{(Formula-4)}$$

ii. Hot-plate test

The hot-plate test employed in this study was as previously described by *Magaji et al.* (2008) with slight modification. Male or female albino mice were fasted overnight with water given *ad libitum*. A hot-plate apparatus (UGO BASIL 7280, Germany) was used for determining the analgesic effect of morphine and the plant extract. The temperature was maintained at 55° ± 1°C. Each mouse was placed on the hot-plate in order to obtain its response to electrical heat induced nociceptive pain stimulus. Licking of the paws or jumping was taken as an indicator of the animal's latency of nociceptive response to heat-induced nociceptive pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was taken (reaction time). A cut-off period of 45 sec was observed to avoid damage to the paws. The animals which showed forepaw licking or jumping response within 15 secs were selected for this study 24 h prior to the experiment. Each mouse served as its own control. Before treatment, its reaction time was taken thrice at 30 min interval. The mean of these three determinations constituted initial reaction time before treatment of the mouse. The mean reaction time for the groups was pooled to obtain the final control mean reaction time (Tb). Each of the mice in the group was thereafter treated with either TW80 (0. 15 mL/10 g, P.O.), morphine (10 mg/kg, s.c) (M10), and plant extract (250 mg/kg (RM250), 500 mg/kg (RM500) or 1000 mg/kg (RM1000), P.O.). The reaction time of each mouse was again evaluated at 30 (except morphine where measurement was taken 15 min after administration), 45, 60, and 90 min after treatment. This was pooled for the mice in each treatment group and the final test mean value for each treatment group at each measurement was calculated. This final test mean value represented the after treatment reaction time (Ta) and was subsequently used to determine the percentage thermal pain stimulus or protection by applying Formula – 5.

% Protection against thermal stimulus =

$$\frac{\text{Test mean (Ta)} - \text{Control mean (Tb)}}{\text{Control mean (Tb)}} \text{ X } 100 \qquad \text{(Formula-5)}$$

3.5.5. Acute Toxicity Study

Two groups of each 10 albino mice, 5 male and 5 female, weighing 25 to 35 g were formed. Food was withdrawn 16 h before the start of the experiment *(Martin-Herrera et al., 2007)* with water available *ad libtum*. Then, the mice in group I and II were orally treated with 5000 mg/kg of aqueous (RA5000) and 80% methanol (RM5000) extracts of the plant, respectively. Each mouse was observed for symptoms of toxicity for the following 15 days in terms of mortality, behavioral changes and weight loss *(Martin-Herrera et al., 2007)*.

4. RESULTS

4.1. Phytochemical Screening

The present study carried out on the plant samples revealed the presence of medicinally active constituents. Both aqueous and 80% methanol extracts of rhizomes of *R. abyssinicus* were found to be positive for the presence of phenolic compounds, tannins, saponins, flavonoids, terpenoids, steroids, anthraquinones and cardiac glycosides. However, the crude extracts were devoid of alkaloids.

4.2. Diuretic Activity: Effect on Urine Volume

4.2.1. Aqueous Extract

Groups of mice treated with the aqueous extract of *R. abyssinicus* increased diuresis clearly in a dose dependent manner (Table 1). RA500 increased urine volume by 43.8% starting from the third hour ($P < 0.05$) and maximum effect (45.5%) was observed at the fifth hour ($P < 0.05$) compared to controls (Table 1). Mice treated with RA750 significantly increased diuresis at the second, third, fourth hours ($P < 0.05$) and an effect (52.7%) was observed at the fifth hour ($P < 0.01$) (Table 1). However, the group of mice treated with the highest dose, RA1000, produced significant increase ($P < 0.01$) in urine volume starting from the second hour (50%) to the fourth hour (60%) and maximum increase of urine volume (96.4%, $P < 0.001$) was observed at the fifth hour (Table 1). Fr10 produced significant diuresis ($P < 0.001$) starting from the first hour after its administration and continued in the same manner for the whole period of the experiment (Table 1).

Although the plant extract showed lower effect than that of the standard drug, Fr10, RA1000 was able to produce a comparable diuretic effect at the fifth hour. The diuretic activity of RA1000 was also very close to that of Fr10 (0.92 vs 1) (Table 1).

Table1: Effect of aqueous extract of the rhizomes of *Rumex abyssinicus* Jacq on diuresis in mice (n=8)

Group	Volume of Urine (mL)					Diuretic action	Diuretic activity
	1h	2h	3h	4h	5h		
DW	0.63 ± 0.13	1.15 ± 0.14	1.2 ± 0.14	1.37 ± 0.13	1.37 ± 0.13	1.0	
Fr10	1.6 ± 0.15^{a3}	2.43 ± 0.09^{a3}	2.6 ± 0.09^{a3}	2.88 ± 0.15^{a3}	2.95 ± 0.15^{a3}	2.15	1.0
RA500	0.78 ± 0.15^{b3}	1.38 ± 0.10^{b3}	$1.73 \pm 0.13^{a1,b3}$	$1.95 \pm 0.15^{a1,b3}$	$2 \pm 0.15^{a1,b3}$	1.45	0.68
RA750	0.85 ± 0.09^{b2}	$1.6 \pm 0.05^{a1,b3}$	$1.83 \pm 0.09^{a1,b3}$	$2.03 \pm 0.17^{a1,b2}$	$2.1 \pm 0.17^{a2,b2}$	1.53	0.71
RA1000	0.98 ± 0.11^{b1}	$1.73 \pm 0.11^{a2,b3}$	$1.93 \pm 0.11^{a2,b2}$	$2.2 \pm 0.09^{a2,b1}$	2.7 ± 0.10^{a3}	1.97	0.92

^{a}*: against control,* ^{b}*: against standard,* ^{1}*:* $P<0.05$*,* ^{2}*:*$P<0.01$*,* ^{3}*:*$P<0.001$

4.2.2. 80% Methanolic Extract

The effect of the 80% methanolic extract of *R. abyssinicus* on urine volume was also dose dependent (Table 2). The smallest dose of the extract, RM250, significantly increased urine volume (28.1%, $P<0.05$) at the third hour. RM500 significantly increased diuresis throughout the experiment ($P<0.05$ at the first hour, $P<0.01$ at the second, third and fourth hours and $P<0.001$ at the fifth hour) (Table 2). A much higher effect was also observed with RM750 ($P<0.01$ at the first hour and $P<0.001$ from the second to fifth hour). Fr10 showed significant effect ($P<0.001$) throughout as compared to controls (Table 2).

RM250 and RM500 produced significantly lower effect on urine volume than Fr10. However, RM750 produced comparable effect on diuresis to that of Fr10. The fact that RM750 showed such a comparable effect to that of Fr10 was also strengthened by their closer diuretic activities (0.98 vs 1) (Table 2).

Table 2: Effect of 80% methanol extract of the rhizomes of *Rumex abyssinicus* Jacq on diuresis in mice (n= 8)

Group	Volume of Urine (mL)					Diuretic Action	Diuretic Activity
	1h	2h	3h	4h	5h		
TW80	0.65 ± 0.09	1.25 ± 0.07	1.43 ± 0.09	1.57 ± 0.09	1.57 ± 0.09	1	
Fr10	1.57 ± 0.12^{a3}	2.27 ± 0.32^{a3}	2.3 ± 0.12^{a3}	2.43 ± 0.11^{a3}	2.85 ± 0.07^{a3}	1.81	1
RM250	0.9 ± 0.13^{b2}	1.49 ± 0.15^{b3}	$1.83 \pm 0.12^{a1,b2}$	$1.93 \pm 0.05^{a1,b3}$	$1.93 \pm 0.06^{a1,b3}$	1.22	0.68
RM500	1.15 ± 0.11^{a1}	$1.83 \pm 0.05^{a2,b1}$	1.98 ± 0.07^{a2}	$2 \pm 0.07^{a2,b2}$	$2.43 \pm 0.08^{a3,b2}$	1.54	0.85
RM750	1.23 ± 0.96^{a2}	1.97 ± 0.05^{a3}	2 ± 0.04^{a3}	2.18 ± 0.06^{a3}	2.8 ± 0.09^{a3}	1.78	0.98

^{a}: against control, ^{b}: against standard, ^{1}: $P<0.05$, ^{2}: $P<0.01$, ^{3}: $P<0.001$

4.3. Diuretic Activity: Effect on Urinary Electrolyte Excretion

4.3.1. Aqueous Extract

The effects of different doses of aqueous extract of rhizomes of *R. abyssinicus* and Fr10 on 5 h urinary electrolyte (Na^+, K^+, and Cl^-) excretion in mice are presented in Table 3. Mice treated with the extract increased urinary electrolyte excretion in a dose dependent manner. RA500 significantly elevated (*$P<0.05$*) urinary excretion of Na^+, K^+ and Cl^- by 28.7, 63.1 and 49.8%, respectively as compared to controls. It was also depicted that the group of mice treated with RA750 produced a significantly higher urinary electrolyte excretion of Na^+ (32.6%, *$P<0.01$*), K^+ (87.6%, *$P<0.001$*) and Cl^- (56.9%, *$P<0.01$*) as compared to controls. The highest effect of the extract was also observed with RA1000. This dose was able to increase urinary electrolyte excretion of Na^+, K^+, Cl^- by 54.9, 93.6 and 79.1%, respectively as compared to controls. On the other hand, Fr10 showed a significantly higher urinary excretion (*$P< 0.001$*) of Na^+ (86%), K^+ (94.5%), and Cl^- (84.1%) ions as compared to controls.

All the doses of the extract produced significantly lower excretion of urinary Na^+ than Fr10 (*$P<0.001$* for RA500 and RA750 and *$P<0.01$* for RA1000) (Table 3). However, the excretion of K^+ and Cl^- was found not to be significantly different between the extract and the standard. Table 3 also shows that the saluretic indices of Na^+, K^+, Cl^- of the extract at the highest dose, RA1000, and Fr10 were closer to each other (1.55, 1.94, 1.74 vs 1.86, 1.94, 1.84) (Table 3). It was also observed that the Na^+/K^+ ratio of Fr10 was higher than that of the extract (Table 3).

Table 3: Effect of aqueous extract of the rhizomes of *Rumex abyssinicus* Jacq on 5h urinary electrolyte excretion in mice (n=8)

Group	Urinary electrolyte concentration (mmol/L)			Saluretic Index*			Na/K
	Na^+	K^+	Cl^-	Na	K	Cl	
DW	60.13 ± 4.75	28.51 ± 1.65	63.75 ± 2.46				2.11
Fr10	111.85 ± 2.90[a3]	55.44 ± 4.68[a3]	117.38 ± 5.24[a3]	1.86	1.94	1.84	2.02
RA500	77.38 ± 3.86[a1,b3]	46.5 ± 3.09[a1]	95.5 ± 5.77[a1]	1.29	1.63	1.50	1.66
RA750	79.75 ± 2.67[a2,b3]	53.49 ± 4.21[a3]	100 ± 4.14[a2]	1.326	1.875	1.57	1.49
RA1000	93.13 ± 3.58[a3,b2]	55.2 ± 4.54[a3]	114.13 ± 11.90[a3]	1.55	1.94	1.79	1.69

[a]: against control, [b]: against standard, [1]: P<0.05, [2]:P<0.01, [3]:P<0.001

**Saluretic Index=mmol of electrolyte of test group/mmol of electrolyete of control group*

4.3.2. 80% Methanolic Extract

As shown in Table 4, 80% methanolic extract of the plant enhanced urinary electrolyte excretion (Na^+, K^+ and Cl^-) in a dose dependent manner. RM250 produced significantly higher urinary electrolyte excretion of Na^+ (47.1%, *P<0.05*), K^+ (81.5%, *P<0.05*) and Cl^- (52.3%, *P<0.01*) as compared to controls. The result also showed that RM500 significantly increased urinary excretion of Na^+, K^+ and Cl^- by 63.7% (*P<0.01*), 89.5 % (*P< 0.01*) and 60% (*P< 0.01*), respectively. The highest dose of the extract, RM750, produced significantly higher urinary electrolyte excretion of Na^+ (78.9%, *P<0.001*), K^+ (99.5%, *P<0.01*), and Cl^- (76.3%, *P< 0.001*). Fr10, however, shown to increase urinary electrolyte excretion of Na^+, K^+, Cl^- by 89.7% *(P<0.001)*, 99.5% *(P<0.01)*, and 84.6% *(P<0.001)*, respectively as compared to controls.

The 80% methanolic extract produced saluretic effect comparable to that of the standard drug. Only RM250 showed significantly lower effect *(P<0.05)* on Na^+ urinary excretion than Fr10. On the other hand, the urinary electrolyte excretion profile of the extract and Fr10 did not significantly differ in case of K^+ and Cl^-. Similar with the aqueous extract, the saluretic indices of the urinary Na^+, K^+, Cl^- excretion of RM750 and Fr10 were much closer to each other (1.79, 1.99, 1.76 vs1.89, 1.99, 1.85) (Table 4). The present study also revealed that the Na^+/K^+ ratio of Fr10 was higher than that of the 80% methanolic extracts (Table 4).

4.4. Electrolyte Content of the Extract

In the case of aqueous extraction method, water soluble salts could be present in the extract and subsequently interfere with the urinary excretion. The content of Na^+, K^+ and Cl^- in the aqueous extract was, therefore, determined. The result showed that the Na^+ and Cl^- content of aqueous extract of *R. abyssinicus* at all doses tested was non detectable by the instrument used in the present study. The K^+ content of the plant extract was also found to be very small (19.6, 20.3, and 45 mmol/L for RA500, RA750 and RA1000, respectively).

Table 4: Effect of 80% methanol extract of the rhizomes of *Rumex abyssinicus* Jacq on 5h urinary electrolyte excretion (n=8)

Group	Urinary electrolyte concentration (mmol/L)			Saluretic Index*			
	Na^+	K^+	Cl^-	Na	K	Cl	Na/K
TW80	59.25 ± 3.81	27.67 ± 1.73	63.38 ± 2. 87				2.14
Fr10	112.38 ± 2.94^{a3}	55.19 ± 4.67^{a2}	117.0 ± 7.33^{a3}	1.89	1.99	1.85	2.04
RM250	87.13 ± 6.76^{a1,b1}	50.23 ± 6.01^{a1}	96.5 ± 5.19^{a2}	1.47	1.81	1.52	1.73
RM500	96.97 ± 8.82^{a2}	52.44 ±6.07^{a2}	101.38 ± 4.60^{a2}	1.64	1.89	1.60	1.85
RM750	106 ± 6.68^{a3}	55.19 ± 4.21^{a2}	111.75 ± 10.08^{a3}	1.79	1.99	1.76	1.92

^{a}: against control, ^{b}: against standard, ^{1}: P<0.05, ^{2}:P<0.01, ^{3}:P<0.001

**Saluretic Index=mmol of electrolyte of test group/mmol of electrolyete of control group*

4.5. Analgesic Activity

4.5.1. Acetic acid-Induced Writhing Test

The effects of methanolic extract and aspirin on acetic acid-induced writhing are presented in Table 5. All doses of the extract reduced acetic acid-induced writhing significantly and dose dependently. Accordingly, the smallest dose of the extract, RM250, ablated writhing by 26.31% (*P< 0.01*). RM500 and RM1000 also reduced the number of writhing by 60.26% and 67.55%, respectively (*P<0.001*). The standard drug, ASA100, had inhibited acetic acid-induced abdominal constriction significantly (72.4%, *P<0.001*) compared to controls.

It was also noted that the plant extract, RM250, produced significantly lower effect (*P<0.001*) than ASA100 on acetic acid-induced writhing in mice. However, the effect produced by RM500 and RM1000 were not significantly lower than the effect produced by ASA100 (Table 5).

Table5: Effect of 80% methanol extract of the rhizomes of *Rumex abyssinicus* Jacq on acetic acid-induced writhing in mice

Group	Number of writhing per 20 min.	Percentage inhibition (%)
TW80	38 ± 1.98	
ASA100	10.5 ± 1.69^{a3}	72.36
RM250	$28 \pm 1.00^{a2,b3}$	26.31
RM500	15.17 ± 1.76^{a3}	60.26
RM1000	12.33 ± 1.09^{a3}	67.55

^{a}: against control, ^{b}: against standard, ^{2}:P<0.01, ^{3}:P<0.001

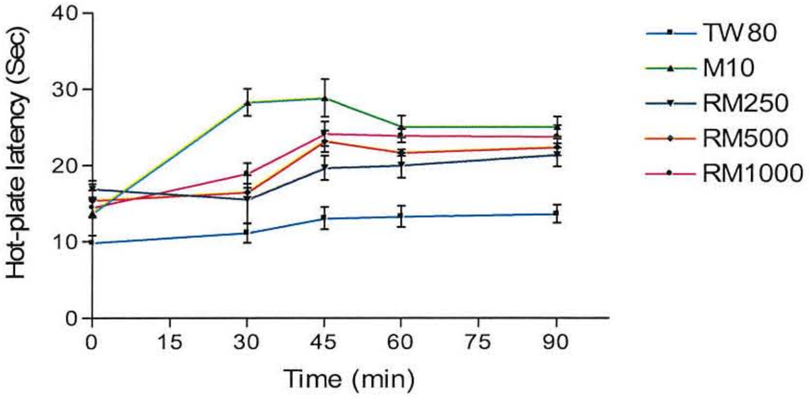

Fig.4.1. *Effects of 80% methanol extract of Rumex abyssinicus* Jacq *at different doses and morphine on hot-plate test. TW80= Tween 80 (4%); M10= Morphine 10 mg/kg; RM250, RM500, RM1000 = 80 % Methanol Extract of R. abyssinicus Jacq at doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg, respectively.*

Table 6: Effect of 80% methanol extract of the rhizomes of *Rumex abyssinicus* Jacq on hot- plate test in mice (n=6)

Group	Hot plate latency(sec) 30min	45min	60min	90min
TW80	11.17 ± 1.27	13.1 ± 1.46	13.32 ± 1.40	13.66 ± 1.17
M10	28.25 ± 1.78^{a3}	28.82 ± 2.45^{a3}	25.05 ± 1.43^{a3}	25.05 ± 1.31^{a3}
RM250	15.55 ± 3.16^{b3}	19.68 ± 1.59^{b2}	20.00 ± 1.60^{a2}	21.37 ± 1.51^{a2}
RM500	16.48 ± 0.59^{b2}	23.15 ± 1.41^{a2}	21.67 ± 0.42^{a3}	21.37 ± 1.51^{a2}
RM1000	18.95 ± 1.32^{a1,b1}	24.15 ± 1.59^{a2}	23.9 ± 0.93^{a3}	23.75 ± 1.47^{a3}

^{a}: against control, ^{b}: against standard, ^{1}: P<0.05, ^{2}:P<0.01, ^{3}:P<0.001

Table 7: Effect of 80% methanol extract of the rhizomes of *Rumex abyssinicus* Jacq on % protection against hot-plate stimuli in mice

Group	% protection against hot-plate stimuli			
	30min	45 min	60min	90 min
TW80	-20	-5.09	-3.523	-1.1
M10	104.66	108.72	81.48	81.48
RM250	12.65	42.6	44.89	54.74
RM500	19.41	67.71	56.96	62.03
RM1000	37.28	74.96	73.15	72.06

4.6. Acute Toxicity

Mice were observed for 15 days to see if aqueous and 80% methanolic plant extracts have acute toxicity in mice. Neither the aqueous nor the 80% methanolic extracts of the rhizomes of *R. abyssinicus* used in the tests produced acute toxicity. This had been evidenced by the absence of lethargy, tremor, fatigue, paralysis, loss of weight and autonomic behavioral changes. There was also no mortality observed in the study period.

5. DISCUSSION

In the present study, the extracts of the rhizomes of *R. abyssinicus* were orally administered in mice to evaluate the diuretic and analgesic effects as rhizome is the part of the plant used traditionally in different parts of Ethiopia.

Decoction was employed as a method of extraction to simulate the traditional preparation. The herbalists recommend boiling of the plant material with water to prepare it for treatment of hypertension. The solubility profile of the active component (s) of the plant may vary. The bioactive ingredient (s) in the plant responsible for the claimed diuretic and/or analgesic activities may not sufficiently be extracted by the decoction method if they are less soluble in water. 80% methanol was therefore used for extraction, in addition to the decoction method, to see if changing a solvent could have any marked effect on the action of the plant.

5.1. Diuretic Activity

Diuresis has two components: increase in urine volume (water excretion) and a net loss of solutes (i.e. electrolytes) in the urine *(Jackson, 2006)*. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream. In the present study, therefore, both of these parameters were measured to evaluate the diuretic effect of the plant extracts.

The reason for using furosemide as a positive control was because of the fact that loop diuretics such as furosemide can dramatically increase the urinary flow rate as well as their strong saluretic effect. They are also one of the most clinically used diuretics for treatment of several ailments *(Jackson, 2006)*.

Previous studies have shown the advantage of pre-treating or "priming" of the test animal with various fluids. As diuretics are employed clinically in the treatment of edema, it would seem to be most important to demonstrate effectiveness in the presence of electrolyte and water *(Nedi et al., 2004)*. Thus, excess water and electrolyte was given to the mice to simulate edema.

Both aqueous and 80% methanol extracts of rhizomes of *R. abyssinicus* showed a clear and significant dose dependent diuresis. Compared with the aqueous extract, the methanolic extract produced better diuretic effect. This difference in their effect could be seen in the different doses used in this study. While the hydroalcoholic extract, RM250, was able to produce significant diuresis in 5 h (Table 2), the same dose of aqueous extract of the plant had been devoid of any appreciable effect that led to its exclusion from the actual experiment. Increasing the dose did not affect the effect produced by the two extracts. For e.g., the diuretic effect produced by RA1000 was still lower to that achieved by RM750 (2.8 ± 0.09 vs. 2.7 ± 0.10 mL) (Tables 1 & 2). Moreover, the diuretic activity (0.98) of RM750, closer to the diuretic activity of Fr10 (1.0), is higher than that of RA1000 (0.92). It is therefore possible to suggest that the ingredient (s) of the plant material responsible for the diuretic activity is probably less polar and hence better extracted in 80% methanol than water.

The diuretic effect of the plant extracts was generally high and qualitatively similar to that of Fr10. Although the lower doses of the extracts produced diuretic effect significantly lower than Fr10, both RA1000 and RM750 of the aqueous and methanolic extracts, respectively, were able to produce effects comparable to that of Fr10 at the fifth hour (Table 1& 2) which clearly shows that the extract has the potential to induce diuresis markedly as those of known synthetic diuretics like furosemide.

The effect of both aqueous and methanolic extracts of *R. abyssinicus* on diuresis was accompanied by marked increases in urinary Na^+, K^+ and Cl^-. Their effect on electrolyte excretion was significant and dose dependent as compared to controls. The extracts, especially at higher doses, showed electrolyte excretion profile qualitatively similar to that of Fr10 although the Na^+/K^+ ratio of Fr10 was higher than that of the extracts. The extracts are also unlikely to be acting as thiazide diuretics, as these diuretics relatively increase the urinary K^+ level more and alter the urinary Na^+/K^+ ratio *(Ratnasooriya et al., 2004)*. In this study, however, both urinary Na^+ and K^+ levels were more or less similarly increased.

Furosemide increases urinary flow rate and urinary excretion of sodium, potassium and chloride, by inhibiting Na^+–K^+–$2Cl^-$ symporter in the thick ascending loop of Henle and inhibiting carbonic anhydrase enzyme *(Rose, 1991; Shinkawa et al., 1993; Puscas et al., 1999)*. Aqueous and methanolic extracts of the rhizomes of *R. abyssinicus* produced effects on diuresis, and urinary excretion of Na^+, K^+ and Cl^- similar to that of furosemide, albeit at a lower potency due to the crude nature of the extract. Although no mechanistic studies had been performed in this work, given the similar diuretic and saluretic activity of the extracts and furosemide, it is plausible to assume that the active component (s) of the rhizomes of *R. abyssinicus* could have a furosemide-like action. It is therefore possible that the extract of *R. abyssinicus* exerted its diuretic activity by inhibiting tubular reabsorption of water and accompanying ions, as such action has been hypothesized for some plants (*Pantoja et al., 1991; Bevevino et al., 1994*).

It is also well known that potassium overloading, which occurs when the kidney tubules are incapable of absorbing it, produces urinary excretion of the osmotic type. Quantitative determinations of the ions present in the aqueous extract of *R. abyssinicus* revealed the presence of very low amounts of potassium salts. This suggests that diuretic effect does not seem to be an osmotic type, as K^+ content of the extract was low to account for the diuretic effect, a result seems to be in line with other plant extracts reported *(Caceres et al., 1987; Sripanidkulchai et al., 2001; Mart'ýn-Herrera et al., 2007; Aissaoui et al., 2008)*.

Regarding the 80% methanol extract, it should be pointed out that water soluble salts could not be present in the extract in sufficient amount as they have poor solubility in such solvents. Hence, such water soluble solutes do not subsequently interfere with the urinary excretion, in contrast to the aqueous extract which causes a removal of these salts. Thus, the notable diuretic effect produced by the methanol extract reaffirmed the notion that the diuretic activity of *R. abyssinicus* was not due to its content of potassium salt rather it was due to intrinsic ability of the plant to exert the effect.

The effect of the extracts on urinary electrolyte excretion was also observed clearly in proportion to the water excretion effect of the plant which again supports the idea that the diuretic effect of *R. abyssinicus* was of the saluretic type in contrast to aquaretic type typical of most phytodiuretic agents (*Mart'ýn-Herrera et al., 2007).*

As it was emphasized, diuretic properties of extracts of *R. abyssinicus* could be due to active principles of the plant. Previous studies have revealed that flavonoids *(Jouad et al., 2001)*, caffeic acid derivatives *(Olah et al., 2003)*, susqueterpene lactones, triterpines, coumarins and carotenoids *(Hopps et al., 1967; Twaij et al., 1983; Olah et al., 2003)*, tannins, saponins and organic acids *(Abed and Benmarbet, 1981)* have diuretic activities. Preliminary phytochemical analysis of this study revealed that tannins, saponins, flavonoids, terpenoids and steroids are the constituents of the plant. Therefore, these natural constituents might be acting synergistically or individually to exhibit the diuretic effects of the plant. It is also possible that the extracts might manifest cumulative effect of several substances in the extract and/or due to secondary active metabolite (s) (*Tanira et al., 1988*). However, the exact component (s) of the plant responsible for the diuretic and saluretic activities remains to be seen.

Despite strong diuresis and saluretic effect, the extract was not associated with a reduction in urinary K^+ levels which suggests that the plant is not acting as potassium-sparing diuretic unlike some other plant extracts reported to have an interesting K^+-saving effect *(Martin-Herrera et al., 2007)*. This fact may point out that the extract exhibited no advantageous effect in respect to hypokalemia, a potential adverse effect of furosemide. However, loop diuretics are the most powerful of all diuretics. They are clinically used in patients with salt and water overload due to a host of conditions such as pulmonary oedema, heart failure ascites, and hypertension. Loop diuretic mode of action of the extract indicates that *R. abyssinicus* might be a useful alternative to furosemide and other loop diuretics in the treatment of such conditions. Moreover, the onset of the diuretic action of the extract such as RM750 was sufficiently rapid and had a fairly long duration of action as it produced its significant effect from the first hour *($P<0.01$)* to the fifth hour *($P< 0.001$)* of the experiment. This is an appealing diuretic profile as it would curtail the frequency of administration despite an increased risk of hypokalaemia as with other therapeutically used loop diuretics.

5.2. Analgesic Activity

The second part of this work aimed to investigate the analgesic effect of 80% methanol extract of rhizomes of *R. abyssinicus* at different doses. Previously reported study on antiinfflamatory effect of the plant was performed by using 80% methanol for the extraction process *(Getie et al., 2003)*. Assuming that the plant constituents having anti-inflammatory effects probably produce analgesic effect as well, similar solvent was preferred for extraction of the active materials of the plant for analgesic study in the present work.

The analgesic activities were evaluated by two mouse models, which could provide response to two different grades of noxious stimuli including thermal stimulus and chemically induced tissue damage *(Zhou et al., 2008)*. Acetic acid-induced writhing test was used for detecting peripheral analgesia *(Deraedt et al., 1980)*. It is very sensitive and able to detect antinociceptive effects of compounds at dose levels that may appear inactive in other methods like tail flick test *(Bentley et al., 1981)*. However, the test is not specific as it does not indicate whether the activity was central and/or peripheral *(Chan et al., 1995)*. Although it is recognized that the acetic acid writhing test is a useful measure of analgesic activity, the method often shows positive responses even to non-analgesic compounds such as central nervous system depressants *(Hendershot and Forsaith, 1959)*. The intraperitoneal injection of acetic acid produces an abdominal writhing response due to sensitization of chemo-sensitive nociceptors by prostaglandins (*Sutharson et al., 2007*). Increased level of prostanoids, particularly PGE_2 and $PGF_{2\alpha}$ *(Derardt et al., 1980)* as well as lipoxygenase products *(Dhara et al., 2000)* have been found in the peritoneal fluid after intraperitoneal injection of acetic acid. Aspirin is a nonselective inhibitor of both COX isoforms and hence prostaglandin production. It is also most effective in reducing pain of mild to moderate intensity through its effects on inflammation and because it probably inhibits pain stimuli at a subcortical site *(Jackson, 2006; Harlan, 2007)*. Together with its availability, this was the reason for using this drug as reference drug in the acetic acid-induced writhing test.

All the doses of 80% methanol extract of rhizomes of *R. abyssinicus* reduced acetic acid-induced writhing in mice in a dose dependent manner. The result of the present study also depicted that the effects produced by the plant extract at RM500 and RM1000 (60.3% and 67.6% of percentage inhibition of writhing, respectively) were not significantly different from the effect produced by ASA100 (72.36% inhibition) on acetic acid-induced writhing in mice showing that the plant extract may have comparable effect on acetic acid-induced writhing test with that of ASA100.

As mentioned above, the abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptive receptors to prostaglandins. The analgesic effect of the extract may therefore be due either to its action on visceral receptors sensitive to acetic acid, to the inhibition of the production or action of algogenic substances such as prostaglandins or the inhibition at the central level of the transmission of painful messages.

Previous studies showed that 80% methanol extract of rhizomes of *R. abyssinicus* produced anti-inflammatory effect by inhibiting the synthesis of PGE_2 *(Getie et al., 2003)*. The coexistence of analgesic and anti-inflammatory activities is well defined for various NSAIDs, particularly salicylates and their congeners *(Famaey, 1983)*. The principal therapeutic effects of NSAIDs derive from their ability to inhibit prostaglandin G/H synthase (COX) which convert arachidonic acid to the unstable intermediates PGG_2 and PGH_2 and leads to the production of thromboxane A_2 and a variety of prostaglandins *(Burke et al., 2006)*. Prostaglandins are also known to cause pain *(Roberts and Morrow, 2001)* and NSAIDs are particularly effective when inflammation has caused sensitization of pain receptors to normally painless mechanical or chemical stimuli *(Burke et al., 2006)*. It is of interest therefore, that the extract behaved probably similar to the NSAIDs and thus correlates well with the ethnomedical use of the plant in painful and inflammatory conditions. There are several speicies which have previously been reported to have such dual analgesic and anti-inflammatory effects in agreement with our result *(Idid et al., 1998; Shanmugasundaram and Venkataraman, 2005; Yonathan et al., 2006; Panthong et al., 2007; Magaji et al., 2008)*.

saponins were some of the constituents identified in the extract of *R. abyssinicus* by the present phytochemical screening. Therefore, the analgesic effect of the 80% methanolic extract may be due to the presence of these plant constituents. The constituents may produce analgesic activity by acting either singly or in combination. However, further studies are needed to isolate the active constituent (s) responsible for the observed effect and to reveal the possible mechanisms of action of the analgesic activity of the plant.

5.3. Acute Toxicity

It was revealed that the plant did not produce acute toxicity in mice even at larger dose (5000 mg/kg) of both aqueous and 80% methanolic extracts. The absence of acute toxicity confirmed the safe nature of the ingestion of this plant since doses which seem clearly higher than the typically used dosage in folk medicine failed to elicit any toxic symptoms in mice. This result suggests that the LD_{50} of the plant is higher than 5000 mg/kg.

6. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the aqueous and 80 % methanolic extracts of the rhizomes of *R. abyssinicus* have been confirmed to exert diuretic activity notably a saluretic type. Therefore, the activity was not due to an osmotic mechanism related to the salts contained within the plant. The diuretic profile of the extracts also exhibited similarity to that of furosemide. Findings also strongly revealed that 80% methanol extract of the rhizomes of *R. abyssinicus* has both peripheral and central analgesic activity. The extract was very safe when taken orally as both the aqueous and 80% methanolic extracts failed to exhibit overt toxicity, in the rodent model. Therefore, findings of the present study illustrated that correlations exist between the popular ethnobotenical use of the plant and genuine diuretic and analgesic activities of the rhizomes of *Rumex abyssinicus*.

However, no study was performed to confirm the exact mechanism (s) and site (s) of action of the diuretic and analgesic activities of the plant extract and thus remains to be clarified in further studies. Also, considerable attention should be given to identify the potent fractions or exact active component (s) of the plant extract responsible for the diuretic and analgesic activities; their exact roles as well.

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Declaration

I, the undersigned, declare that this thesis is my original work and had not been presented for a degree in any other University.

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