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ABSTRACT

***Objectives*: To determine the serum electrolytes levels in animals treated with *Catha edulis* leaf extract.**
***Design*: Experimental study**
***Setting*: Department of Biological Sciences at the Chepkoilel University College, Moi University**
***Subjects*: Fourteen (14) reproductively mature and healthy small East African male goats (Capra aegagrus hircus) from Kerio Valley in Rift Valley province of Kenya.**
***Intervention*: Plasma electrolytes (Na^+, K^+, Ca^{2+} and Cl^-), urea, creatine and glucose levels were investigated in eight control and fourteen experimental small East African male goats before and after treatment with *Catha edulis* leaf extracts. At the end of the study the animals' were sacrificed and their kidneys extracted for histological examination.**
***Results*: mean sodium and calcium levels were lower in treated animals than non-treated animals (138 v/s 143.6 mmol/L and 3.3 v/s 2.2 mmol/L respectively), $p<0.001$ Plasma glucose levels also declined from 4.0-4.1mmol/L to 3.3-3.5mmol/L following the *Catha edulis* leaf extract treatment. However serum nitrogenous metabolites levels increased significantly in *Catha edulis* treated animals (urea; 6.5 v/s 5.2 mmol/L and creatine; 69.9 v/s 55.4 mmol/L). Histological examination of renal tissue of *Catha edulis* treated animals revealed degenerative changes and hypercellularity in the glomeruli as well as interstitial inflammatory cell infiltration. Nuclei of proximal convoluted tubule cells also appeared pyknotic while those of the macula densa appeared granular.**
***Conclusion*: The present study showed that *Catha edulis* treatment was associated with electrolyte imbalance which may have been as a result of degenerative changes in the renal system. The findings are a pointer to the fact that *Catha edulis* use may predispose the users to renal disorders and subsequent electrolyte imbalance.**

INTRODUCTION

Catha edulis (Khat) is an evergreen shrub that is grown around the Red Sea and on the Eastern coast of Africa (1) and is estimated to have five to ten million regular users worldwide. Khat chewing is localised in the source countries since only their fresh leaves have the potential to produce the desired effects. Fresh leaves of *Catha edulis* are mainly taken orally to induce a state of euphoria and subjective well-being which are attributed to its cathinone constituent (2, 3). Tolerance to khat develops with regular use and its stimulating and euphoric effects have been shown to provide a strong inducement for the user to obtain daily supplies and engage in regular khat chewing (4). Khat users consequently develop psychic or physical dependence or both with continued use of the substance (5). Due to its medical and socio-economic

impact on the society the World Health Organization (WHO) has classified khat as a substance of abuse although the source countries maintain an ambiguous legal position on khat (6).

Major socio-economic problems have been attributed to knat use in many countries (3, 4). Reports show that khat affects the cardiovascular, digestive, respiratory, endocrine, hapatobiliary and genitourinary systems (1). Studies on rabbits demonstrated short and long term toxic effects of khat leaves on the liver as evidenced by significant increases in plasma levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (2, 4, 5). In other studies on rats, the administration of khat caused a decline in the liver enzymes, ALP and increased the activities of acid phosphatase, lactated dehydrogenase (LDH) and also increased the total bilirubin (7). Histopathological changes have been reported in both liver and kidneys of rats treated with khat (2, 8).

However, contradicting findings have been elucidated concerning hepato-renal toxic effects produced by regular khat chewing in humans (4). Certain studies have however shown damage to kidneys with decline in total serum, elevated urea and creatine levels associated with regular human khat chewing. The present study was therefore designed to determine the toxic effects of intravenously administered khat on goats which being herbivorous regularly use shrubs, some of which are of the same class as khat. Goats were chosen for this study because they are mammals and the mammalian renal physiology is the same in all members of the Phyla. Therefore, the findings can be used to depict the physiological and histological responses in human users of the plant. Although most studies have used primates as models for human diseases, goats have also gained acceptance as an established model for biomedical research and for surgical training and teaching. They are used in medical, orthopedic, psychological, chemotherapeutic and physiologic research (9). In addition, comparatively goats are less expensive, easy to handle and transport, affectionate, friendly and clean, and they appear to be hardier than other members of the ruminant family (10). The present study analyzed kidney histology and monitored levels of plasma electrolytes and proteins in treated goats to outline associated patho-physiologic changes to administration of khat.

MATERIALS AND METHODS

Choice and Maintenance of Research Animals: Fourteen (14) reproductively mature and healthy small East African male goats (Capra aegagrus hircus) from Kerio Valley in Rift Valley province of Kenya were used in the present study. The animals were aged between 8-12 months and weighed between 10-20kg. The animals were purchased and transported to the department of Biological Sciences at the Chepkoilel University College where they were housed in a fly-free-pen and allowed four weeks of acclimatisation. The animals were exposed to free range grazing at the University College farm with water and salt lick provided ad libitum. On arrival at the campus each animal was subjected to a preventive pneumonia antibiotic dosage (procaine penicillin, Unisel Pharma (Kenya) Ltd) and multivitamin treatment to boost protection against microbial infections. During the acclimatization period the animals were dewormed once (Valbazen, Ultravetis, East Africa LTD; dosage, 4mls/10-20Kg) and sprayed with an acaricide (Ectomin) manufactured to control ectoparasites.

Experimental Design: This hypothesis testing experimental study was designed to investigate the effects of intravenously administered *Catha edulis* extracts on specific blood physical and biochemical parameters. After the acclimatisation periods, blood samples were obtained from each animal twice a week for a period of four weeks which was regarded as pre – treatment phase of the study. After the pre-treatment period, the animals were randomly assigned into two groups, experimental group (8) and control group (6). The experimental goats received single daily dose of 120 mg/kg body weight of *Catha edulis* extracts through the jugular vein while the control goats received corresponding amounts of normal saline. The intravenous route of drug administration was chosen in this study to help evade the detoxifiers found in the goats' gastrointestinal system (11). Blood was sampled twice weekly for laboratory analysis of electrolytes, metabolites, protein and glucose levels, during the six weeks administration *Catha edulis* extracts. At the end of the treatment period four representative experimental animals and two controls were sacrificed and their kidneys harvested for histological examination.

Preparation of Catha edulis Extract: *Catha edulis* extracts were obtained from fresh bundles of (1.5 kg; stem tips and leaves) the plant purchased from the local venders in Eldoret Town. Aluminum foil was used to cover the leaves in order to avoid light-induced disintegration of the active ingredients (12). The leaves were chopped into pieces on a glass plate, weighed and then crushed with pestle and mortar and the extract obtained filtration, evaporation and distillation processes following an overnight stay in methanol as described by Nilesh's group (12). The distillation process took about 4 hours and was considered complete when the entire methanol had evaporated. The resultant extract was weighed and the volume determined. It was then stored at -20°C under the cover of aluminum foil until required for administration during which the extract was

reconstituted using normal saline. Each animal received 120 mg/kg body weight of the *Catha edulis* extract through the jugular vein (adjusted to a final volume of 3ml in normal saline) by use of a 21gauge needles mounted on 10 ml syringe.

Blood Samples Collection, and determination of plasma electrolytes and protein metabolites levels: Five milliliters (5ml) of whole blood was collected twice weekly from each goat by jugular puncture into sterile vacutainers using a 21 gauge needle mounted on a 10ml syringe. Each vacutainer with blood was shaken gently before being placed into a tubes' rack in a cool box and then transported to the lab where the samples were processed for serum electrolytes(Na^+,K^+,Ca^{2+} and Cl^-), protein metabolites (urea and creatine) using an automated analyser (Reflectron Automated Analyser, Beckman, U.S.A) and glucose levels using a hemocue (Angelhom, Sweden).

Kidneys harvesting and tissue processing: Each harvested kidney was first rinsed in phosphate buffered saline, placed in a fixative (10% formalin) and then refrigerated at - 70° C until required for processing. On the processing day the tissues were dehydrated in ascending grades (50%, 70%, 90%, 95% and 100%) of alcohol and embedded in paraffin wax in the automatic tissue processor. Sections of 5µm thickness were cut in HM 310 rotary microtone and mounted on Mayer's egg albumin coated glass slides. Each section was dewaxed in two changes of xylene for two minutes then rehydrated through descending grades of alcohol for thirty minutes (100%, 95%, 90%, 70%, and 50%) and further washed in tap water. The gradual removal of water from the tissues was to avoid sudden shrinkage of the cells and secondly to avoid the rapture of the cells. The sections were then stained with Harris haematoxylin and counterstained with eosin (1% for 2min) according to Mallory's method (13). The sections were then dehydrated in ascending grades of alcohol for thirty minutes (70%, 80%, 95%, and 100%), cleared in three changes of xylene and mounted in DPX and examined microscopically. The images (Mg x 400) of the tissues were projected into a sensitive digital camera fitted onto the microscopic eyepiece. The camera captured digitized field images (Mg x 400) that were fed into a computer for quantitative image analysis and printing. The photo micrographs were displayed for comparison with normal tissue sections.

Data Analysis Procedure: Inferential statistics was used in analyzing data. Results were expressed as the mean value – SEM. Statistical differences between groups were assessed by student's t test. Values of $p<0.05$ were considered significantly different.

RESULTS

Mean serum sodium levels in animals that did not receive the *Catha edulis* treatment ranged between 143.3±1.2 -144±0.6 mmol/L whereas following in animals on *Catha edulis* treatment serum sodium decline to a mean of 138±0.33 mmol/L (Table 1). Intravenously administered *Catha edulis* extract therefore was associated with serum sodium electrolyte imbalance leading to significant decline ($p<0.001$). Mean serum calcium levels also declined significantly from pre-treatment mean level range of 3.2±0.05-3.3±0.06 mmol/L to 2.2±0.03 mmol/L after six weeks of *Catha edulis* leaf extract (120 mg/Kg body weight) treatment ($p<0.002$). Mean serum potassium and chloride levels in non-treated animals ranged from 4.5±0.09-4.6±0.15 mmol/L and 105.5±0.06-106.9±0.07 mmol/L, respectively. In animals that received six weeks of *Catha edulis* leaf extract (120mg/Kg body weight) treatment, mean serum Potassium (4.6±.08 mmol/L) and Chloride (104.4±0.0 mmol/L) were not significantly different from the levels observed in non-treated animals (Table 1).

Table 1

Plasma electrolyte levels in the small East African goats before and during treatment with Catha edulis leaf extract (120mg/Kg body weight)

Serum electrolytes	Pre–treatment		Post–treatment	
	Controls	Experimental	Controls	Experimental
Na^+ (mmol/L)	143.6±0.8	143.3±1.2	144.0±0.6	138.3±0.33^
K^+ (mmol/L)	4.6±0.06	4.6±0.15	4.5±0.09	4.6±0.08*
Cl^- (mmol/L)	106.9±0.7	106.9±0.7	105.5±0.6	104.4±0.9*
Ca^{2+} (mmol/L)	3.3±0.06	3.3±0.05	3.2±0.05	2.2±0.03^

^Pre-treatment and post-treatment values are significantly different ($P<0.05$).
*Pre-treatment and post-treatment values are not significantly different ($P>0.05$).

Animals treated with *Catha edulis* leaves' extract (120mg/Kg body weight) treatment also tended to manifest raised serum urea and creatinine. Mean urea levels in treated animals (6.5±0.10 mmol/L), was higher than in non-treated animals (5.1±0.08-5.3±0.09 mmol/L) (Table 2). This increase was however not statistically significant. Creatinine levels on the other hand increased significantly from pre-treatment range of 55.1±0.36 -56.1±1.2mmol/L to 69.9±1.5 mmol/L after the six weeks of daily *Catha edulis* leaves' extract treatment ($p<0.001$) (Table 2). These results showed that *Catha edulis* leaves' extract treatment have the capacity to disrupt the metabolism of proteins in the body.

Table 2

Plasma urea and creatine levels in the small East African goats before and during treatment with Catha edulis leaves' extract

Serum nitrogenous	Pre– treatment		Post– treatment	
metabolites	Controls	Experimental	Controls	Experimental
Urea (mmol/L)	5.2±0.03	5.1±0.08	5.3±0.09	6.5±0.10*
Creatine (μmol/L)	55.4±2.05	55.1±0.36	56.1±1.2	69.9±1.5^

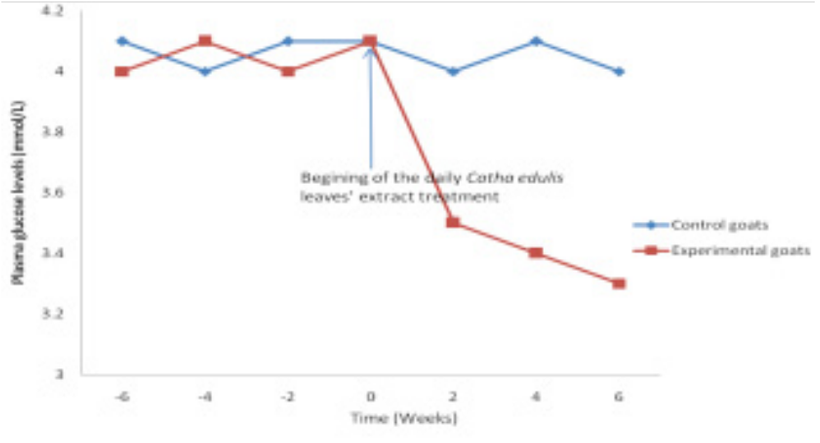
* Pre-treatment and post-treatment values not significantly different

^ Pre-treatment and post-treatment mean values significantly different ($P<0.05$).

In the non-*Catha edulis* leaves' extract-treated goats the mean blood glucose level ranged between 4.0±0.05-4.1±0.07mmol/L (Figure 1). Following the daily *Catha edulis leaves'* extract (120 mg/Kg body weight) treatment the mean fasting blood glucose level continually declined to 3.5±0.06 mmol/L during the second week, 3.4±0.05mmol/L in the fourth week and 3.3±0.04 mmol/L during the sixth week (Figure 1). Therefore, treatment with *Catha edulis* extract was associated with progressive hypoglycemia.

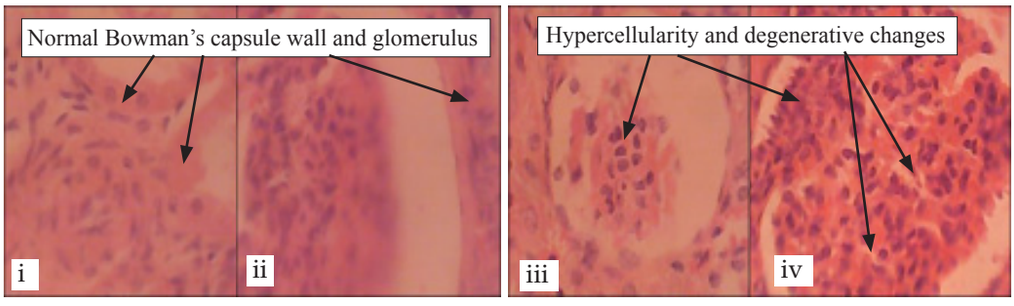
Figure 1

Plasma glucose levels in male goats before and during treatment with Catha edulis leaves' extract



Kidney histology of representative control goats exhibited a thick normal Bowman's capsule wall and glomerulus cells (Figure 2, i and ii). On the other hand histology of animals that received daily treatment of *Catha edulis* leaves' extract (120mg/Kg body weight) showed kidney tissues disruption with thin walled Bowman's capsule, hypercellularity and degenerative changes within glomeruli (Figure 2, iii and iv).

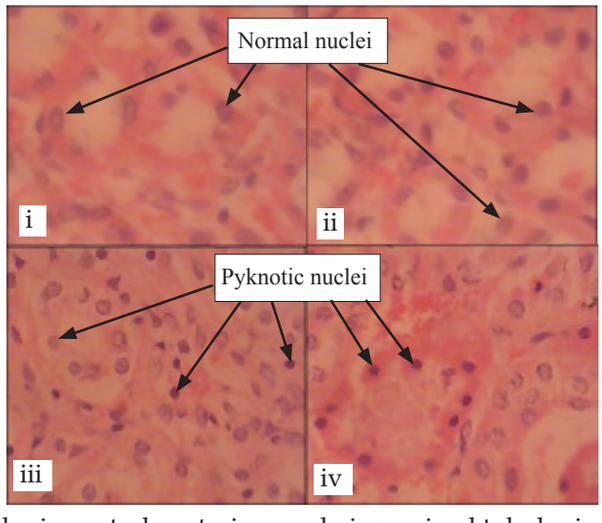
Figure 2
Histology of the kidney



(i) Bowman's capsule cells and (ii) the glomerulus cells in normal male goats; (iii and iv) the glomeruli in the male goats after two months of *Catha edulis* leaves extract (120 mg/Kg body weight) treatment

In non- *Catha edulis*-treated animals, proximal convoluted tubule appeared normal (Figure 3, i-ii) and afferent arterioles appeared well structured with normal thickness. In experimental animals on the other hand, proximal convoluted tubules exhibited cells with pyknotic nuclei (Figure 3, iv-vi). Nuclei of macula densa cells were also granulated and there was interstitial inflammatory cell infiltration. These changes denoted disrupted physiology of fluid and electrolytes that could have affected filtration and re-absorption across the renal functional unit.

Figure 3
Histology of the kidney tubules



i, ii and iii - proximal tubules in control goats; iv, v and vi- proximal tubules in treated goats

DISCUSSION

Physiological and biochemical stability of blood constituents especially electrolytes, proteins, nitrogenous metabolites, glucose and hormones are under the regulation of the body's homeostatic mechanisms mediated at the kidneys. Although fluctuations in electrolytes and other metabolite levels are expected under normal circumstances, these must be within ranges that can still support optimal body physiology. Electrolytes and metabolites in particular are very important in determining the body fluids' osmotic potential which is one of the main forces behind fluid exchanges between the different body fluid compartments. Therefore, the level of fluid exchange between the intracellular and extracellular fluid compartments of the body is dependent on the concentrations of osmotically active substances in the blood. The renal system is key to the homeostatic processes of the body since it is endowed with the capacity to filter, reabsorb and secrete blood components depending on the body's osmotic needs. This study has demonstrated that daily intravenous infusion of *Catha edulis* leaf extract caused hyponatremia, hypocalcaemia, hypoglycemia and a disruption of kidney tissues in small East African male goats. The plasma levels of urea and creatine were however elevated.

Under normal conditions the reabsorption of Sodium ions is mediated by the renin-angiotensin-

aldosterone mechanisms at the distal part of the nephron. In response to acute or chronic depletion of sodium ions the regulatory mechanisms are usually stimulated to increase the plasma levels of aldosterone (13). The control mechanisms involve sensors within macula densa cells followed by secretion of renin from granular cells. This study observed degenerative changes in the glomeruli of the kidneys following daily intravenous infusion of *Catha edulis* leaf extract. The kidney cells also exhibited pyknotic nuclei in the proximal convoluted tubules, and granular nuclei in the Juxtaglomerular apparatus. These findings are a pointer to the fact that the administration of *Catha edulis* leaf extract to the goats induced tissue disruption in the renal functional tissue. This may have disrupted the osmotic potential sensors thereby probably accounting for the altered electrolytes and nitrogenous metabolites' balance. Since the juxtaglomerular apparatus which consist of the macula densa, mesangial and juxtaglomerular cells is the site of renin secretion the degenerative changes reported in this study possibly disrupted the renin-angiotensin-aldosterone system functioning. These findings suggest that the renal tissues' degenerative changes caused a failure in osmotic potential change detection, renin and aldosterone secretion which may have resulted into lack of sodium ions reabsorption from the renal filtrate and hence the hyponatremia. Renal reabsorption usually handles sodium ions with glucose through a cellular symporter system. Under the system sodium ion reabsorption occur together with that of glucose at the proximal convoluted tubule segment. The observed hypoglycemia following the daily intravenous infusion of *Catha edulis* leaf extract may be attributed to disruption of sodium-glucose reabsorption as a result of renal tissue degenerative changes. In other studies, hypoglycemia has be attributed to the delayed glucose absorption from the intestines due to the phenolic action of tannins and inorganic ions in the *Catha edulis* leaf extract (8, 14). The present findings highlight the possible role of impaired tubular sodium and glucose reabsorption in causation of systemic imbalance in both.

Under normal circumstances the loss of sodium ions is concomitantly accompanied with potassium retention. In this study the potassium ion levels in the experimental animals was only slightly higher than in the control animals during the treatment phase although the levels were not statistically significant. It is highly possible that presence of *Catha edulis* realted compounds in circulation and renal tubules disruption caused failure in potassium ion reabsorption. On the other hand, hyponatremia may have precipitated inter-compartmental compensatory electrolyte exchange between the intracellular and extracellular fluid via Na/KATPase pump, leading to extrusion of sodium from cells and pooling of potassium within them. Inability to normally metabolize potassium by disrupted renal tubular tissues could also have played a role in observed potassium outcomes.

The reduced plasma calcium ion levels following the daily intravenous infusion of *Catha edulis* leaf extract was suggestive of disrupted calcium ion homeostasis. Since degenerative changes in the kidney were observed in this study, it is highly possible that this may have impaired calcium ion re-absorption at the renal tubules. Most ions cross cell membranes through pumping or ion channel mechanisms. Degenerative changes in the renal tubules tissues may have caused disruption in the calcium ion channels and the calcium ion pumping mechanisms.

Renal dysfunction was further confirmed by the elevated levels of plasma urea and creatine. Healthy kidneys are expected to filter urea and other waste products which are excreted through urine. High blood urea nitrogen levels are usually attributable to renal dysfunction, high protein intake, inadequate fluid intake or poor circulation. Uremia can also be due to increased urea production and poor renal clearance which may be associated with reduced urine flow (15, 16). *Catha edulis*-induced urinary system disorders have been previously reported (17) and there are indications that *Catha edulis* chewing can predispose individuals to urinary system disorders. Some studies have also reported khat-induced cell injury that lead to impaired excretion of urea and creatine (1, 18). Elevated plasma levels of urea and creatine have been linked to renal diseases that are associated with reduced ability to excrete these nitrogenous metabolites (19). Effects of raised urea levels include bone marrow suppression, platelet dysfunction, and altered mental state all of which can lead to serious health condition. .

This study has demonstrated that the use of *Catha edulis* leads to electrolyte imbalance. Underlying causative mechanisms associated with use of *Catha edulis* could include osmotic activity of cathinone and its induced degenerative changes at the renal tubules. Therefore, chewing *Catha edulis* leaves has potential health risks to the user more particularly in case of high dosages and prolonged use.

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