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EFFECTS OF *CATHA EDULIS* FORSK (KHAT) AND ASCORBIC ACID ON SERUM ELECTROLYTES IN SWISS ALBINO MALE RATS
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ABSTRACT

Background: ***Catha edulis*** **Forsk (Khat) is an evergreen shrub widely consumed in East African countries. Ascorbic acid has been taken as a protective agent in patients with different diseases. However, little is known about the effect of khat and ascorbic acid on serum electrolytes. The aim of this study was to investigate the subchronic effects of khat and ascorbic acid on serum electrolytes in rats.**

Materials and Methods**: A total of 36 adult (7-8 weeks) male Swiss Albino rats (213 -229g weight) were used. The rats received khat extract (ke) 100 mg/kg, 200 mg/kg and 300 mg/kg b.w), khat juice (2.5 ml/kg), ascorbic acid (AA 200 mg/kg) and Tween 80 in distilled water (T80W- 2% v/v) for twelve weeks. Serum electrolytes were measured using Cobas 6000. The data were analyzed using SPSS version 21.0.**

Results: **The mean serum sodium level in rats received ke 100 mg/kg ($p < 0.01$), ke 200 mg/kg ($p < 0.001$), ke 300 mg/kg ($p < 0.001$) and khJ 2.5 ml/kg ($p < 0.001$) was significantly less than in rats received T80W. The serum calcium level was less in rats received ke 200 mg/ kg ($p < 0.05$) and 300 mg/kg ($p<0.05$). The serum sodium level in rats which received ke 200 mg/kg ($p<0.01$) and ke 300 mg/kg ($p<0.05$) were significantly less than in rats which received ascorbic acid.**

Conclusions: **khat induced sodium and calcium imbalance, but not ascorbic acid. Effects of khat on acid-base balance and arginine vasopressin should be investigated.**

INTRODUCTION

Catha edulis Forsk (Khat) is an evergreen shrub grown and widely consumed in East African countries including Ethiopia [1]. A study reported that khat has adverse effects on body systems [1]. Khat users consequently develop psychiatric disorders and are aggravated by khat chewing[2]. Its medical and socio-economic burdens are increasing [1]. However, the prevalence of chewing khat is still high [3].

Some of its adverse effects are related to electrolyte imbalance [4]. However, no studies have been conducted for the subchronic effects of khat on the serum electrolytes in an animal model. Ascorbic acid has been taken as a protective agent in patients with different diseases [5, 6]. However, little is known about its effects on serum electrolytes. The aim of this study was, therefore, to investigate the subchronic effects of khat extract, khat juice and ascorbic acid on the serum electrolytes in male Swiss Albino rats.

MATERIAL AND METHODS

Chemicals: Diethyl ether and chloroform (Siga-Aldrich, Germany), Tween 80, pentobarbital, ascorbic acid, and 70% ethanol purchased from local suppliers in Ethiopia were used in this study.

Plant materials collection: Bundles of fresh khat leaves (9kg) were collected from Aweday, Eastern Ethiopia. The plant specimen was identified, and the voucher number (October 16, 2018, AA002) was given and deposited at the National Herbarium of Ethiopia.

Plant material extraction: After the edible parts of the leaves were separated and washed with tap water, the leaves were freeze-dried at -20^{O}C for 2 days and crushed using a mortar and pestle[7]. Two hundred grams of freeze-dried crushed leaves were placed into a conical flask wrapped with aluminum foil. 400 ml of diethyl ether and chloroform (3:1 v/v ratio) was added into the flask. The mixture was shaken under the dark condition for 48 hours at 20^{O}C using a rotary shaker (New Brunswick Scientific Co, USA) with a speed of 120 rpm. Filtration was made using cotton gauze followed by grade I Whatman filter paper (Cat No 1001 150). The organic solvents were removed through evaporation using Rota-vapor under controlled temperature (36^{o}C), pressure (240 Pascal negative pressures) and rotation with 120 rev/min. Water in the extract was removed through lyophilization and the dry extract was obtained. The khat juice (khJ) was prepared from 12 gm/kg b.w of fresh leaves using T80W. The fresh leaves within the T80W were crushed using a blender machine. The juice was then squeezed and filtered using the gauze and grade I Whatman filter paper. The amount of T80W used to extract the given weight of the leaves and dry extract was determined based on the total weight of each rat and standard vehicle volume.

Animal preparation: A total of 36 adult male white albino rats (7-8 weeks and 213 - 229g weight) were used. Three rats per cages under natural light and dark (12:12hrs) cycles at room temperature were housed. Water and standard pellet diet were available and were *ad libitum*. Rats were weighed twice a week to ensure appropriate dosing. All the studies were conducted under the guidelines for animal care and use [8]. The research was approved by the Physiology Department Research and Publication committee. Then, it was approved by the Institutional Review Board (IRB) Committee of Addis Ababa University.

Grouping and dosing: Rats were randomly assigned into six groups (n= 6/group) and

received T80W, khat extract (ke) 100 mg/kg, 200 mg/kg and 300 mg/kg, ascorbic acid (AA 200 mg/kg) and khat juice (khJ 2.5 ml/kg). The test substances were administered for twelve weeks. The T80W was used as a vehicle through which the khat extract and ascorbic acid were dissolved.

Volume determination and administration: Fresh khat extract, ascorbic acid, khJ, and vehicle were prepared every day. The dose of the extract administered in each rat was calculated based on the total b.w of each rat. The appropriate volume of the vehicle (2.5 ml/kg b.w) was used to determine the amount of volume used to dissolve the calculated dose of khat extract and AA. Each rat in a given group received a single daily oral dose of vehicle, khat extract, khJ, and AA. The final volume for each rat was 1ml and all substances were administered orally using a metal gavage needle. The control group of rats received the same volume of Tween 80 in distilled water.

Blood collection: The procedure used in the previous research [9] was applied in our study. Briefly, the rats were anesthetized using sodium pentobarbital (1ml/kg b.w) before blood sample collection. Five milliliters (5 ml) of over-night fasting blood was collected and put into plain tubes. The blood was collected by cardiac puncture. Then, the tubes were allowed to clot and then centrifuged at 6000 rpm for 10 minutes. The supernatant was slowly transferred into new Eppendorf tubs for sodium (Na^+), potassium (K^+), calcium (Ca^{++}) and chloride (Cl^-) analysis using Cobas-6000.

Statistical analysis: The statistical analyses were done using SPSS version 21.0. Differences in electrolytes between groups were analyzed using one-way ANOVA followed by post hoc analysis. An independent t-test was also used.

RESULTS

The independent t-test showed that rats received khat had significantly less serum Na^+ ($t_{(28)} = 6.24$, $p<0.001$) and Ca^{++} ($t_{(28)} = 2.94$, $p<0.01$) level than rats received T80W. Significant differences in K^+ ($t_{(28)} = -.748$, $p > 0.05$) and Cl^- ($t_{(28)} = .727$, $p > 0.05$) level was not found between the groups. Only serum Na^+ level was significantly less in rats received khat ($t_{(7.72)} = 3.29$, $p<0.05$) than in rats received AA (Figure 1).

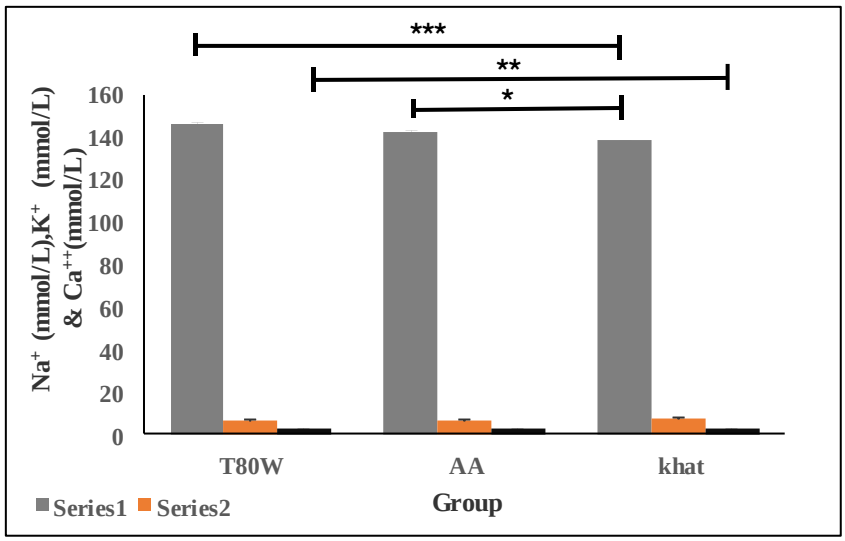

Figure 1: *Effects of khat and AA on serum Na^+, K^+ and Ca^{++} (mmol/L) level. The bars represent mean ± SEM of these electrolytes in rats (n= 6-24 /group) received T80W, AA and khat. ***P < 0.001, **P < 0.01 and *P < 0.05 when the three groups were compared with to each other. T80W = Tween 80 in distilled water and AA = Ascorbic acid.*

One way ANOVA analysis showed that significant differences were observed in serum $Na^+(F_{(5,30)} = 12.17, p < 0.001)$, K^+ ($F_{(5,30)} = 2.99$, $p < 0.05$) and Ca^{++} ($F_{(5,30)} = 3.32, p < 0.05$) level and Na^+ / K^+ ratio ($F_{(5,30)} = 5.54, p<0.01$), whereas significant difference in serum Cl^- ($F_{(5,30)} = 0.78, p>0.05$) level was not observed between groups. The mean serum Na^+ level in rats received ke 100 mg/kg ($p<0.01$), ke 200 mg/kg ($p<0.001$), ke 300 mg/kg ($p<0.001$) and khJ 2.5 ml/kg ($p<0.001$) were significantly less than in rats received T80W (**Table 1**). Rats received ke 200 mg/ kg ($p<0.05$) and 300 mg/kg ($p<0.05$) had significantly less serum Ca^{++} level than rats received T80W. The mean serum Na^+ level in rats received ke 200 mg/kg ($p<0.01$) and ke 300 mg/kg ($p<0.05$) was significantly less than in rats received AA 200 mg/kg. The K^+ level in rats received ke 300 mg/kg was non-significantly increased compared with rats received T80W and AA 200 mg/kg (**Table 1**). The ratio of sodium-to-potassium was also significantly lower ($p<0.01$) in rats received ke 300 mg/kg (table 1).

Table 1

Effects of khat and ascorbic acid on serum electrolytes in rats

Group	Electrolytes (mmol/L), M±SEM				
	Na+	K+	Ca++	Cl^-	Na^+/K^+
T80W	145.67±1.31	5.88±.44	2.78±.23	101.59±.77	25.55±2.06
AA	142.17±1.08	5.99±.97	2.41±.07	101.08±1.55	26.33±3.32
ke 100 mg/kg	140.50**±.99	4.28±.45	2.37±.04	102.43±.95	34.29±2.89
ke 200 mg/kg	136.67***±.92	6.74±1.43	2.08*±.19	99.95±.71	23.28±2.94
ke 300 mg/kg	137.17***±.79	9.14±1.22	2.00*±.19	100.26±.68	16.44±2.26**
khJ 2.5 ml/kg	138.67***±.61	6.95±.51	2.43±.05	100.76±1.22	20.49±1.47

*Each value represents the mean ± SEM of electrolytes. ***P <0.001, **P <0.01 and *P <0.05 when each group of rats was compared with rats received T80W.*

DISCUSSION

In this study, all doses of khat extract and khat juice reduced the serum sodium level significantly compared to the vehicle. A similar finding was observed in the previous study [10]. However, another study indicated that khat extract didn't show significant serum sodium change [9]. The dissimilarity between these findings could be attributed to the extraction protocol and duration of administration. In the previous study, the organic solvent used to extract the khat leaves was methanol and the extract was administered to rats for four weeks. However, the diethyl ether to chloroform (3:1 v/v) ratio and Tween 80 in distilled water were used to extract the fresh khat leaves and rats were administered with the extract for twelve weeks in our study.

Amphetamine, cathinone in khat-like substance, also reduced the serum sodium level [1]. The analogous effect of khat extract and amphetamine on the serum sodium level could be attributed to the similarity between cathinone in khat and amphetamine in structure and pharmacodynamics.

The reduction in the serum sodium level observed in our study could be attributed to the dopamine, renal and liver effects of khat [2, 9, 10, 12 and 13]. Intrarenal dopamine increments enhanced Na^+ excretion and dopamine facilitate the secretion of arginine vasopressin (AVP) [14 and 15]. It has been known that AVP increases water retention and dilutes the blood. This dilution effect of AVP could reduce the serum sodium level.

On the other hand, the liver problems attributed to khat [3] could induce systemic vasodilation and facilitate the secretion of AVP. As mentioned earlier, AVP dilutes the blood and could reduce the serum sodium level. The gastrointestinal effect of khat and excessive water intake during khat chewing [13] could also reduce the serum sodium level. The tannic acid and toxic metals in khat leaves are expected to affect the gastrointestinal function and influence mineral absorption.

Like the previous study [10], the serum calcium level was significantly reduced in this study by khat extract at the middle and higher doses. However, the khat juice didn't show any significant effect on the serum calcium level. This indicated that the extraction process could have effects on the components in the khat leaves. This could alter the serum calcium level. The khat juice was not prepared using organic solvents; rather 2% Tween 80 in distilled water was used to extract the juice.

As it has been shown in the previous study, serum calcium level was significantly less in patients with depression [6]. At the same time, depression is one of the consequences of chronic khat consumption [3]. Therefore, if khat extract reduced the serum calcium level and contributed to depression, its effect on depression could be through its consequence on the serum calcium level.

Oppositely, a study on humans showed that the serum calcium level was significantly higher in normal subjects and diabetic patients who chewed khat [17]. The discrepancy could be attributed to the study subjects used to evaluate the effect of khat on the serum electrolytes. The duration of administration and dose of khat consumption could also be attributed to the discrepancy.

The gastrointestinal effect of khat could be one of the reasons behind the effect of khat on serum calcium level reduction. A study conducted before showed that gastrointestinal inflammation reduced calcium and sodium absorption [18]. Conversely, gastrointestinal inflammation is common among people who

chew khat [13]. Therefore, the reduction in the serum calcium level observed in our study could be attributed to the gastrointestinal inflammatory effect of khat.

Although the difference was insignificant, the serum potassium level was higher in rats received a higher dose of khat extract compared with the vehicle or ascorbic acid. However, it was significantly increased among this group of rats compared with rats which received the lower dose of khat extract. This indicated that there is a probability though which the serum potassium level could be increased if the dose of khat extract was made higher than the maximum dose used in our study and prolonged administration.

Unlike our study, the previous study showed that the serum potassium level was increased significantly by khat extract [10]. The dissimilar finding between these studies could be attributed to the animal species used to evaluate its effect on serum electrolytes. These were African male goats used in the study conducted previously, while the Swiss Albino male rats were used in our study. The solvent used to extract the khat leaves, doses, and duration of administration could also be involved in the discrepancy. The organic solvent used to extract the khat leaves in the previous study was methanol, while the diethyl ether to chloroform (3:1 v/v) ratio was used to extract the fresh khat leaves in our study.

Like our study, a study conducted before indicated that the serum potassium level was increased non-significantly by khat extract administered to rats [9]. However, the dose in the previous study was higher (500 mg/kg) than the maximum dose (300 mg/kg) used in our study. The methanolic extract of khat leaves was administered to rats for four weeks in the study conducted before, while it was for twelve weeks in our study.

The level of serum chloride was not affected by khat extract/juice or ascorbic acid in this study. These findings indicated that the effect of khat extract was more pronounced on the serum sodium and calcium ions than potassium and chloride ions. Although extensive work is required in this area in the future, the psychiatric-like symptoms observed in subjects who chew khat [1] could be attributed to its effect on serum sodium and calcium levels.

In this study, the sodium to potassium ratio was significantly less in rats administered with the higher dose of khat extract. Alternatively, the higher sodium-to-potassium ratio is expected to increase arterial blood pressure. At the same time, hypertension is common among people who chew khat [19]. Therefore, if the sodium-to-potassium ratio was significantly reduced by khat extract observed in our study and if khat increases the blood pressure [19], its effect on the blood pressure could be attributed to its effects on factors other than its effect on the sodium-to-potassium ratio. Therefore, the effects of khat on arterial blood pressure could be through its consequences on the sympathetic nervous system, hemodynamics, and cardiovascular system.

In this study, ascorbic acid didn't show any significant change in the serum sodium, calcium, potassium and chloride levels. A similar finding was observed in the previous study [20]. In the previous study, humans threated with vitamin C didn't show any significant changes in serum sodium, potassium, and chloride ions. However, the serum sodium and potassium levels were increased by vitamin C supplementation in patients with diabetes mellitus [20]. Unlike our study, sodium-to-potassium ratio was

significantly reduced by vitamin C supplementation in both diabetic patients and normal control subjects [20].

CONCLUSION

Khat reduced the serum sodium and calcium ions with non-significant increment in serum potassium ion. However, ascorbic acid administration didn't show any significant effect on serum sodium, calcium, potassium and chloride ions. Effects of khat on acid-base balance and arginine vasopressin need to be investigated in the future.

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