ORIGINAL RESEARCH PAPER
Volume-6 | Issue-11 | November-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

THE ACUTE DOSE- AND TIME- RESPONSES OF CRUDE *CATHA EDULIS* EXTRACT ON SERUM ELECTROLYTES IN MICE

Physiology

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ABSTRACT

This *in vivo* random experimental animal study was conducted to evaluate the acute dose- and time- responses of crude *Catha edulis* extract on Serum electrolytes in mice. A total number of 60 mice were used for this study. The acute dose- and time- responses of crude *Catha edulis* extract on serum electrolytes were measured by ion selective electrolyte analyzer. Serum was collected after the onset of 45, 90 and 180 minutes of oral administration of 100, 200 and 400mg/kg doses. Results showed insignificant variation in Na^+, K^+, Ca^{2+}, and Cl-. The insignificant variation in Na^+, K^+, Ca^{2+}, and Cl- may be related to rapid and sensitive regulatory mechanisms that keeps electrolytes in balance through active and passive transport of ions.

KEYWORDS

Catha edulis, Serum electrolytes, Mice, Electrolyte analyzer

INTRODUCTION

It is estimated that about 20 million people consume khat leaves around the world. Everyday, an estimated six million people chew khat all over the world (Odenwald *et al.*, 2005). Its use traditionally has been confined to the regions where it is grown, because only the fresh leaves have the desired stimulating effects. However, In recent years it becomes more common in western countries due to migration of people from those 13 endemic khat chewing countries like Ethiopia, Somalia and access of air transportation have increased the global distribution of this perishable commodity, and as a result, the plant has been reported in Amsterdam, Australia, Canada, England, Israel, Rome, New Zealand, Wales, and the United States.

khat contains many different compounds, thus, chewing it results dozen of effects. The major effects include on the gastro-intestinal and nervous systems. Constipation, urine retention and acute cardiova scular effects may be regarded as autonomic (peripheral) nervous system effects; increased alertness, dependence, tolerance and psychiatric symptoms as effects on the central nervous system (WHO, 2006). Cathinone is highly responsible for achieving sympatho mimetic effects and CNS stimulation similar to the effects of amphetamine (Schechter et al., 1984) although other less potent stimulant substances namely, norpseudoephedrine (Cathine) and norephedrine are also present (Al- Motarreb *et al.*, 2002). These effects include elevated blood pressure, mydriasis, hyperthermia, anorexia, insomnia, alertness, elevated mood, psychosis, and talkativeness (Mekasha, 1984; Al- Mamary et al., 2002). In addition, other components of khat have their contribution for various clinical complications such as, constipations due to tannin (Halbach, 1972), impotence, respiratory problems (Kennedy et al., 1983). As long as *Catha edulis* has central nerve system stimulating effect, it may induce an increase in the concentration of some major electrolytes in the extra cellular fluid.

Chemically, electrolytes are substances that become ions in solution and acquire the capacity to conduct electricity in the fluid compartm ents of the body. It is because of these dissolved electrolytes and their capacity to conduct current, that it is possible to measure brain activity (Electroencephalography) as well as heart (Electrocardiography) and other muscle (Electromyography) by placing electrodes on the body surface (Horne and Swearingen, 1989).

Electrolytes are found both the intracellular and extracellular fluid in the human body, and the balance of the electrolytes in our bodies is essential for normal function of our cells and our organs. This balance is critically important for things like hydration, nerve impulses, muscle function, and pH level. Maintenance of the internal environment within narrowly restricted limits is an essential characteristic of all mammalian systems. Since electrolytes are chemical compounds that break down into ions, it carrying either a positive or negative charge. When these are not in balance, pathological changes occur in the human body (Lefever *et al*., 2010).

Control is exerted through highly refined and sensitive regulatory systems employing positive and negative feedback methods and functioning chemically or electrically in modifying both cellular and organ level behaviour. Many of the controls are exerted through, by, and upon the blood vascular system. Hence, an indication of the extent of homeostasis, permissible deviations, as well as significant disparities resulting from pathological processes, can be obtained by measuring the physical and chemical attributes of blood. This becomes even more apparent when it is recalled that most somatic cells have a direct dependency upon the fluid constituent of blood for their survival. Plasma is the vehicle for provision of nutrients, removal of excretory products, temperature regulation, and transmission of control information. Thus, electrolytes affect most metabolic processes. The most important electrolytes are calcium, sodium, potassium, and magnesium (Mahan *et al*., 2012).

As long as *Catha edulis* has central nerve system stimulating and pleasurable effect on the basis of the previous study, I postulate that it may induce an increase the concentration of potassium ion and a decrease in sodium, calcium and chloride ions in the ECF.

MATERIALS AND METHODS

Fresh Khat Collection

Six bundles of fresh khat of "Aweday" type were purchased at a local market from one retailer in Bole ,Addis Ababa, who received daily supply from Aweday, its natural habitat, 525 km South of Addis Ababa, Ethiopia. Then these fresh materials of Khat were wrapped in a plastic bundle as chewers do and taken to Ethiopian food and nutrition research institute, for extraction. The fresh materials were washed to remove dust and debris with distilled water.

Extraction

Shoot leaves were collected, and chopped/ crushed with pestle and mortar on a glass plate and weighted (500 grams) by electronic digital balance and placed in Erlenmeyer flasks (≤200g per flask) wrapped with aluminum foil to avoid light induced decomposition. And then ethanol (70%) was added to cover the minced leaves in ratio of 4mL: 1g. Then, put in to rotary /orbital shaker (DS-500) for 24 hrs at the speed of 120rpm at ambient temperature under dark condition. Then filtered the filtrates with Whatman filter paper # 1 and collected and kept in another flask. Rotary evaporator (RE300) is used to remove all traces of ethanol which was used for extraction at a digital water path (RE300DB) at a speed of 3 rotations per second, 40^{0}c temperature and 70kpa vacuum pump (RE3022C) pressure. Finally, the fraction was left overnight in a deep freezer and then lyophilized using freeze dryer (Christ 100400 Bio block Scientific, France) and approximately 40 gram of crude khat extract was yield.

Experimental Animals

Experimental animals (60 male mice) were obtained from Ethiopian food and nutrition research institute, department of Laboratory animal breeding. The animals were housed in mice cages in physiology lab, Department of Physiology, Black Lion Specialized Hospital, Addis Ababa University.

Ion-Selective analyzer

Consist of ion-selective electrodes that detect some specific ions in the presence of other ions and ion concentrations in an analytical solution, typically plasma and serum. Ion-selective analyzers are designed to simultaneously measure electrolyte concentrations of ionized calcium, potassium, chloride, sodium, magnesium and some other ions.

METHODS

Study Design: Laboratory based experiment involving quantitative and descriptive analysis of data.

Study Area: Laboratory of Department of Physiology, Faculty of Medicine, Addis Ababa University; Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia.

Study protocol

A total number of 60 male mice were used for this experiment. The mice were divided in to four categories; one control and three treatment groups (100,200 and 400mg/kg). Different protocols are involved for varieties of acute studies in different methods. The control group (n=6) was administered 0.5ml normal saline while the treatment group (n= 54) were induced one in a manner that depend on time and dose. They divided into 3 main groups (each group of n =18) and administered 100,200 and 400mg/kg *Catha edulis* extract solution for 45 minutes, 90 minutes and 180 minutes and then 1-2mL blood samples were collected from each mouse after the onset of administration through cardiac puncture method. Then, the blood samples were centrifuged in 5000rpm for 3miniutes. Serum was collected in non-heparinized tubes for the analysis of electrolytes. The samples were put in ion selective electrolyte analyzer and the concentrations of sodium, potassium, calcium, and chlorine ions were measured. Each mouse was used only once to avoid the possibility of developing tolerance, drug toxicity or both.

Statistical analysis

All data for electrolytes concentration measurements were expressed as mean ± SE.M. The differences in the mean values of these parameters among groups were analyzed using multivariate ANOVA followed by Tukey's multiple comparison tests. The level of statistical significance was set (at most $p < 0.05$).

RESULTS

The acute dose- and time- response of Catha edulis extract on the Concentration of serum Na^+, K^+, Ca^{2+} and Cl- in mice

As shown in table 1, both dose and on set of acute oral administration of Catha edulis extract had neither direct correlation nor significant change on the concentration of serum Na^+, K^+, Ca^{2+} and Cl- in mice.

Table 1: An acute dose and time response of *Catha edulis* extract on serum concentration of Na^+, K^+, Ca^{2+}, and Cl^- concentration in male mice.

Total number of mice (N=60)	Dose (mg/kg)	Mean ± SE. M ($\frac{mM}{L}$)		
		After 45' (n=6 for each dose)	After 90' (n=6 for each dose)	After 180' (n=6 for each dose)
Groups		[Na^+]		
(CG, n= 6)	0.5ml NS.	150.60 ± 4.16		
(Rx100,n=18)	100mg/kg	152. 00 ± 4.00	151.25 ± 3.95	147.25 ± 2.87
(Rx200,n=18)	200mg/kg	148.60 ± 1.14	151.00 ± 3.60	148.20 ± 3.11
(Rx400,n=18)	400mg/kg	148.30 ± 3.01	147.30 ± 2.99	148.70 ± 2.34
		[K^+]		
(CG, n= 6)	0.5ml NS	10.24 ± 0.15		
(Rx100,n=18)	100mg/kg	10.74 ± 1.07	9.00 ± 0.54	9.28 ± 0.56
(Rx200,n=18)	200mg/kg	10.00 ± 2.44	8.83 ± 1.32	9.20 ± 0.51
(Rx400,n=18)	400mg/kg	8.70 ± 1.81	9.90 ± 1.27	9.60 ± 0.54
		[Ca^{2+}]		
(CG, n= 6)	0.5ml NS	1.13 ± 0.09		
(Rx100,n=18)	100mg/kg	1.04± 0.03	1.10 ± 0.04	1.02 ± 0.10
(Rx200,n=18)	200mg/kg	1.07 ± 0.06	1.06 ± 0.08	1.08 ± 0.03
(Rx400,n=18)	400mg/kg	1.12 ± 0.08	1.14 ± 0.06	1.08 ± 0.01
		[Cl^-]		
(CG, n= 6)	0.5ml NS	117.2 ± 3.89		
(Rx100,n=18)	100mg/kg	120.00 ± 6.05	118.75 ± 2.63	115.00± 1.41
(Rx200,n=18)	200mg/kg	115.20 ± 2.68	118.40 ± 5.07	118.20 ± 1.79
(Rx400,n=18)	400mg/kg	116.50 ± 3.56	112.50± 2.43	118.80 ± 1.64

Values are represented in Mean ± SE.M

CG = control group; NS = normal saline administered grouped mice; Rx100, Rx200, Rx400 = 100mg/kg, 200mg/kg and 400mg/kg *Catha edulis* extract administered grouped mice respectively.

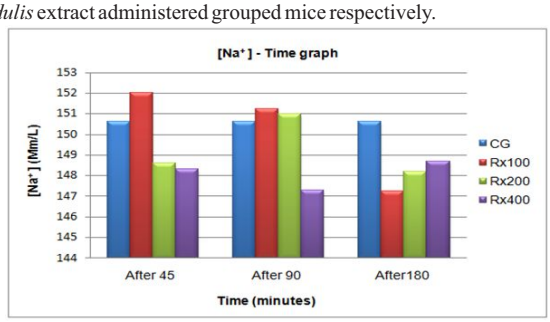

Figure 1: Graph showing the acute dose (100,200 and 400mg/kg) and time (after the onset of 45, 90 and 180 minutes onset of oral administration) response of *Catha edulis* extract solution on serum [Na+] level in male mice. Values are represented in Mean ± SE.M (n=6 for each group).

Rx100 mice had revealed decreasing concentration pattern of [Na^+] (152. 00± 4.00, 151.25 ± 3.95 and 147.25 ± 2.87) as oral administration time increased from 45 minutes to 90minutes, then to 180minutes respectively. However, this alteration in relation to time administered has no any significant change of [Na+] level as compared to mean values of baseline (150.60 ± 4.16). Rx200 mice had slightly higher mean values of [Na+] after 90 minutes (151.00± 3.60) of onset of oral administration than 45 (148.60 ± 1.14) and 180 (148.20± 3.11) minutes. Rx400 mice had slightly higher mean values of [Na+] after 180 minutes (148.70 ± 2.34) than 45 (148.30 ± 3.01) and 90 (147.30 ± 2.99) minutes of onset of oral administration. This showed that as there has no significant correlation between serum [Na+] level with both onset of time and dose of acute *Catha edulis* oral administration. The response shows normal physiological variation. In addition, the variation was irregular and insignificant as compared to mean values of baseline (150.60± 4.16).

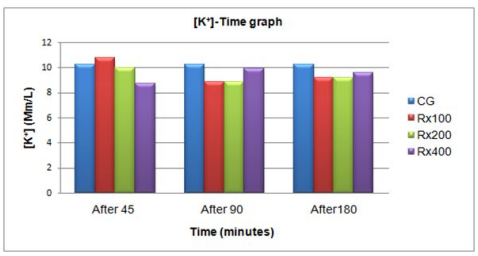

Figure 2: Graph showing the acute dose (100,200 and 400mg/kg) and time (after 45, 90 and 180 minutes onset of oral administration) response of *Catha edulis* extract solution on serum [K^+] level in male mice. Values are represented in Mean ± SE.M (n=6 for each group).

The mean Rx100 (10.74 ± 1.07) mice had shown slightly higher mean [K^+] after the onset of 45 minutes oral administration than 90 (9.00 ± 0.54) and 180 (9.28 ± 0.56) minutes. Similarly, Rx200 mice had shown slightly higher [K^+] after 45 minutes (10.00 ± 2.44) than 90 (8.83 ± 1.32) and 180 (9.20 ± 0.51) minutes. However, Rx400 mice had slightly higher [K^+] after 90 minutes (9.90 ± 1.27) than 45 minutes (8.70 ± 1.81) and 180 minutes (9.60 ± 0.54). This had shown that as there has no correlation between serum [K^+] level and onset of Catha edulis oral administration. The variation had not shown any pattern of alteration and significance as compared to mean values of baseline (10.24± 0.15). This is also true for [K^+] with dose response. It rather had shown normal physiological variation among inter-dose and onset of administration to the K^+ concentration response.

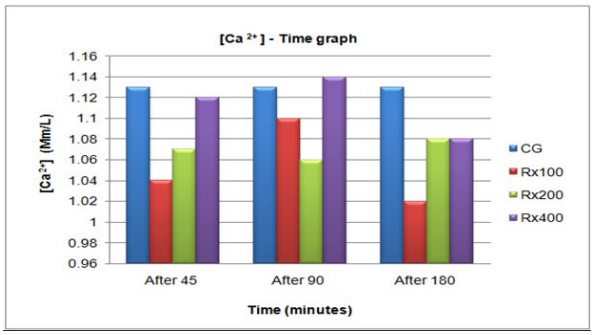

Figure 3: Graph showing the acute dose (100,200 and 400mg/kg) and time (after 45, 90 and 180 minutes onset of oral administration) response of *Catha edulis* extract solution on serum [Ca^{2+}] level in male mice. Values are represented in Mean ± SE.M (n=6 for each group).

The mean [Ca^{2+}] of Rx400 mice (1.12 ± 0.08) had shown slightly higher [Ca^{2+}] than Rx200 (1.07 ± 0.06) and Rx100 (1.04 ± 0.03) mice after the onset of 45 minutes of Catha edulis oral administration. At this time, there had been an increasing pattern of [Ca^{2+}] as does increased. However, this didn't show consistency after 90 and 180 minutes. After 90 minutes onset of oral administration, Rx200 mice had slightly lower mean values of [Ca^{2+}] (1.06 ± 0.08) than Rx100 (1.10 ±0.04) and Rx400 (1.14± 0.06) oral administered mice. After the onset of 180 minutes of oral administration of *Catha edulis*, Rx100 mice had lower mean [Ca^{2+}] (1.02 ± 0.10) than Rx200 (1.08 ± 0.03) and Rx400 (1.08±0.01) mice. This irregular increment and decrement at the onset of 45, 90, and 180 minutes onset of oral administration with the corresponding doses had shown as there were no significant correlation both to dose and time response on the [Ca^{2+}] level.

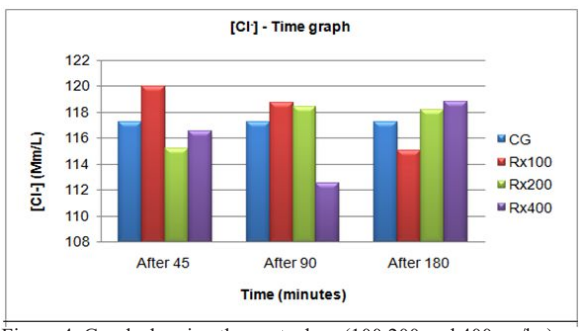

Figure 4: Graph showing the acute dose (100,200 and 400mg/kg) and time (after 45, 90 and 180 minutes onset of oral administration) response of *Catha edulis* extract solution on serum [Cl-] level in male mice. Values are represented in Mean ± SE.M (n=6 for each group).

Rx100 mice had shown slightly higher mean [Cl-] after 45 (120.00 ±6.05) and 90 (118.75 ± 2.63) minutes than after 180 minutes on set of *Catha edulis* oral administration (115.00 ± 1.41). At this dose, there was decreasing pattern of mean [Cl-] as time increased. However, Rx200 mice had shown slightly higher mean [Cl-] after 90 (118.40±5.07) and 180 (118.20 ± 1.79) minutes onset of Catha edulis oral administration than after 45 minutes (115.20 ± 2.68). Similarly, Rx400 mice had slightly higher mean serum [Cl-] after 180 minutes on set of oral administration (118.80 ± 1.64) than 90 (112.50± 2.43) and 45 (116.50 ± 3.56) minutes. This irregular increment and decrement of concentration of serum Cl- after the onset of 45, 90, and 180 minutes with the corresponding doses had shown as there were no significant correlation among time and dose to the serum Cl- concentration level. This variation was irregular and insignificant as compared to mean values of baseline (117.2± 3.89).

DISCUSSIONS

Dose- and time- response of acute oral administration of *Catha edulis* extract had neither direct correlation nor significant change on the concentration of serum Na^+, K^+, Ca^{2+} and Cl- in mice. Even though the serum [Na^+] had shown slightly decrement pattern as dose increased after the onset of 45 and 90 minutes, it had revealed the reverse pattern after 180 minutes. Similarly, serum [K^+] had shown decreasing pattern as dose increased after 45 minutes. Despite this finding, there was no consistency as such in case of post 90minutes and 180 minutes on set of oral administration of *Catha edulis*. So, the acute doses of 100,200 and 400mgkg-1 and time intervals (45, 90 and 180 minutes) of oral administration of *Catha edulis* extract solution had no direct correlation on the effects of serum concentration of K^+. This is also true for serum Ca^{2+} and Cl-.

Even though it had no significance, Rx100,Rx200, Rx400 mice had shown slight decrement in the [Ca^{2+}] in mice as compared to the baseline (from1.13± 0.09 to1.07± 0.05).This may be associated with the vasoconstriction effect of *Catha edulis* extract on vascular smooth muscles, and which is dependent on the ECF Calcium (Ca^{2+}). Some smooth muscles have action potentials that look very much like action potentials in neurons. At the cellular level, however, smooth muscle action potentials are different from those in neurons and skeletal muscle because in smooth muscle, the depolarization phase is due to the entry of Ca^{2+} rather than Na^+. In smooth muscle, an action potential is not required to open voltage-gated Ca^{2+} channels. Graded potentials may open a few Ca^{2+} channels, allowing small amounts of Ca^{2+} into the cell. This Ca2+ ion entry depolarizes the cell and opens additional voltage-gated Ca2+ channels.

CONCLUSION

Results of the present investigation on acute electrolyte study demonstrated that both dose- and time of acute oral administration of *Catha edulis* extract had neither direct correlation nor significant change on the concentration of serum Na^+, K^+, Ca^{2+} and Cl- in mice. Rather, it had shown an irregular slight decrement and increment of concentration in all of these ions in response to dose and on set of administration. It hadn't shown any pattern of alterations. The overall result had shown normal physiological variations. Probably, this type of alteration in the electrolyte profile measurement following *Catha edulis* administration may associate with the variation of age among the experimentally tested mice. The overall result had shown normal physiological variations. Possibly this insignificant change may related with highly refined, rapid & sensitive regulatory mechanisms that keeps electrolytes in balance through active & passive transport of ions as well as with an increase in glial cells function.

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