Effect of khat leaves extract (*Catha edulis*) on the excitability of frog gastrocnemius.

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Abstract

This study was held on the isolated excitable tissue of frog's gastrocnemius muscle. Experiments were down by determining the dose dependent of leaves of khat extract. Work down in accordance with personal presupposition since reported that khat have psycho stimulant effects accordingly we thought khat might have effects on systemically biology that mediated by chticol amines.

Results showed the effect of khat leaves extract on excitability of muscle contraction that was easily attested when the electrical stimuli were applied either through the nerve or directly to the muscle. Whereas khat treatment showed a significant increase in the contraction period of the gastrocnemius muscle and both of the relaxation periods and half relaxation time were shortened. Also it was found that pretreatment by khat leaves extract caused changes in the tension output, increased the height of effect intensity with prolonged the contraction period, while the latent period was decreased and reduced slightly the half relaxation period of the twitch comparison with the normal control muscle. These experimental results indicated that khat had highly stimulant effects on muscle, whereas caused liberation of thermo-enzymatic and chemical energy during contraction which converted into mechanical energy, which manifested its self as work.

Introduction

Khat shrub (Catha edulis) is cultivated and widely chewed by people in Yemen and East Africa, as a regional custom and tradition, and because if it is highly stimulant effects.

Khat is cultivated as coffee in the high valleys, exposed to the bleak of heat of the sun only a few hours, among the semi arid region , varies according to the temperature, related to topography of the region, medium to rainfall and medium to low temperature (Dahmash,1996).

Its height ranges from 6-30 feet and may even obtain height of more that 60 feet (Kennedy, 1982).

Khat leaves and buds are chewed in fresh condition in Yemen, and are used, dried by Yemenis outside the country.

Chewers get an increase in alertness, enhancement perception and energy for few hours after starting khat chewing, then replacement by anorexia and reactive depression. (Hassan, etal, 2003).

The active compound from khat and amphetamine having similar chemical structure-pheny1 alkyl amine (Thabet, 1997). Moreover Cathine and Cathinone are the two main natural compounds of amphetamine like. (Kalix, 1992, Bajupair, 1996, and Al- Aubidy, 1997).

On another hand Reported earlier by Kalix,1987 that khat had shown to have a releasing effect at physiological catecholamine storage sites.

Calcagnetti and Schechter, 1992 reported that khat increases the locomotor's activity, respiratory excitation and mydriasis in frogs injected with khat extract (Mosso,1891).

Some studies showed that khat chewing was associated with keratonic lesions in the mouth, and khat chewers showed more genetic damage in

buccal mucosa cells when compared with non-chewing control. (Kassie,etal,2001) and (Ali,etal,2004).

Khat chewing is recognized as a real national problem in Yemen with adverse consequences for health and socioeconomic development of Yemen country. Although is widely used in pharmacological and chemical investigations have been done on the isolation and identification of the active compounds, but no systemic biological study has been performed.

Material and Methods

Extraction :

Fresh leaves of khat (7gm) were mixed once with (150ml) distilled water and another (7gm) were mixed with (300ml) distilled water both shaked strongly boiled for 15-20 minutes, and then the tow mixtures were filtered and kept in a closed container in the refrigerator till it cools.

The tow cool solutions mixed with methanol and then the mixtures were boiled till the methanol had been evaporated. The cooled solutions were strongly shaken and filtered.

Gastrocnemius preparation of toad :

Thirty five toads *(Bufo melanostictus)* of both sexes weighing between 60-80 gm were throughout the experiments.

Gastrocnemius muscle of one limb of the toads was used as a control. Two identical muscle chambers made it possible to run two experiments simultaneously.

The muscles were removed from its original with attached small length of femu with Achilles' tendon from the pitted animal.

The tendon was hung by a small hook connected by a thread leading to an isotonic recording torsion lever under a fixed tension of 5 gm.

After the excision, the muscle was placed in a 10 ml bath of Ringer solution prepared. After equilibration, the muscle was placed with two spring silver electrodes of minimal tension. All experiments were performed at room temperature (20-25C). (Karmakar,etal,1995,).

Stimulating Techniques:

Stimulating was given through (Grass S44 stimulator providing monophasic square wave shocks through the isolation unit for direct supremaximal stimuli. The stimulus patterns were those, which evoked maximal tension responses when applied directly to the muscle. The parameters of electrical stimulation were tested to ensure that the contractions were always maximal tension response.(Ghai,1995).

Recording of the mechanism:

Inside the smoked kymograph recording drum, the contractions were recorded with the isotonic lever load with fixed tension.

Treatment by khat:

1ml of Khat extract was added to the muscle to produce a concentration of 0.23mg/ml and 0.46mg/ml in 4ml of Ringer Solution. Contraction period and relaxation period of the untreated control muscle were recorded typographically. The same muscle was then balanced in khat extract for 5-10 minutes and replaced in normal ringer solution for recording the contraction kinetics.

The muscle was sewed during repeated stimulation and recording.

Tension output:

By an isotonic myograph of torsion, type with the muscle subjected to supremaximal stimulation strength tension was measured .for analyzing the kymograph in script, a base line was drawn in each case and a line concurred

the maximal slope of the rising and of the falling phase of the twitch was prorogated to the base line in every case. The contraction time for the rising phase was the interval from the point of peak tension and the relaxation time for the falling phase. In order to inter-relate the tension with stimulus frequency, muscle tensions were recorded in response to 30 seconds.(Ghai,1995).

Excitability:

Two indications of the excitability of whole muscle were gauged : the threshold current needed for evoking maximum twitch continue duration of rectangular pulse (20 sec.) and the minimum duration same pulse required for inducting response to a supra-maximal current strength.

Excitability was taken to be inversed to the measured threshold current. The intensity duration curve under normal position was changed by khat treatment.

Work done:

The work done by:

A muscle = weight lifted × distance through which the weight is lifted.

$$= W X (l/L X H).$$

Whereas: W= was the weight of the muscle preparation(=2.700 gm).
l = is the short arm of lever (=4.5 cm.)
L=is the long arm of lever (=20cm.)
H = is the maximum recording height of the peak Tension.

Results

Effects of different concentrations of khat leaves extract on the response of the gastrocnemius muscle to electrical stimulation were tested in a few experiments.

The most effective dose of khat leaves extract for hasting the excitability of other contraction variables was noted to be limited with in khat extract concentrations of 46mg/ml which is representing of dose 0.00066mg/kg and 23mg/ml which consider a dose of 0.00033mg/kg Whereas the different effects between these two doses was very slight, when set for 5-10 minutes.0.00025mg/kg, which there was no change. Table (1).

Excitability of gastrocnemius muscle was increased by khat extract treatment, and the effect of khat on excitability was easily proved when electrical stimuli were applied either through the nerve or directly to the muscle.

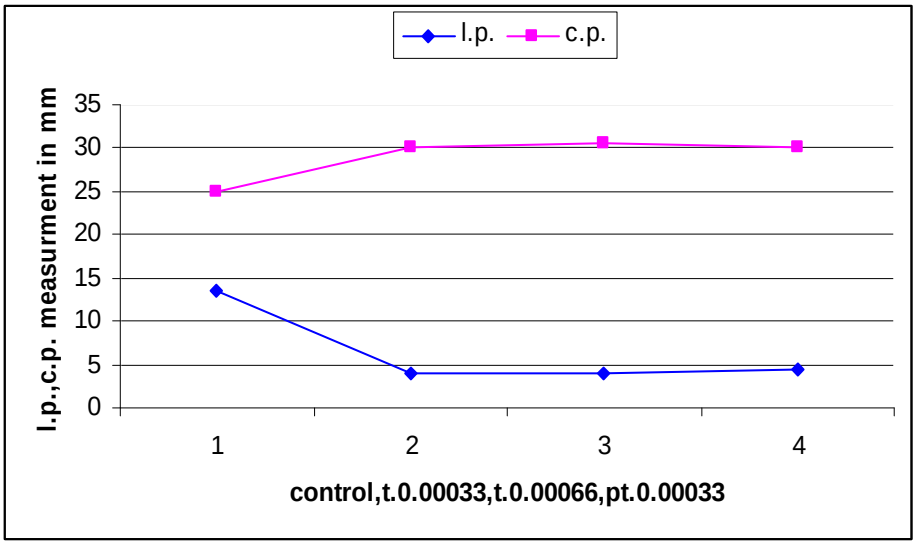
Results showed that Khat treatment effected significant increase in the contraction time of the gastrocnemius muscle(121) of second and the latent period, relaxation period and half relaxation time were shortened(-49, -94,-36) of a second respectively. Also was found that pretreatment by khat extract caused changes in the tension output, prolonged in mm the contraction period(22) and the height of effect intensity(72.7)while the latent period was decreased(-66.7) and reduced the half relaxation period of the twitch (-36) in comparison with the normal control muscle. Figure (2).

The calculation of the mean of work done was(8.50 mm) for the normal control muscle and it was (13.5,and11.24 mm) for the khat extract treated muscles of tow doses of 0.46mg/ml and 0.23mg/ml in succession.

Table (1) : Comparison records of various phases of the muscle contraction, showing the effect of different doses of khat extract on the muscle contraction accordingly to the muscle contraction in normal state. Measurement mean values of muscle contraction's phases were in (mm).

No.	Various phases in muscle Contraction (mm)	Untreated muscles	Treated muscles by doses of khat extract:		Retreated muscles by dose of khat extract:
		Control	23 mg/ml	46 g/ml	23 mg/ml
1-	Latent Period (L.P.)	13.5	4	4	4.5
2-	Contraction Period (C.P.)	25	30	30.5	30
3-	Relaxation Period (R.P.)	26.5	19.5	19.5	19.5
4-	Half Relaxation Period (H.R.P.)	11.75	7.9	7.5	8
5-	Peak Tension (P.T.) or Maximum recording height of the contraction (Mhc)	8.25	12.75	14.25	12.25

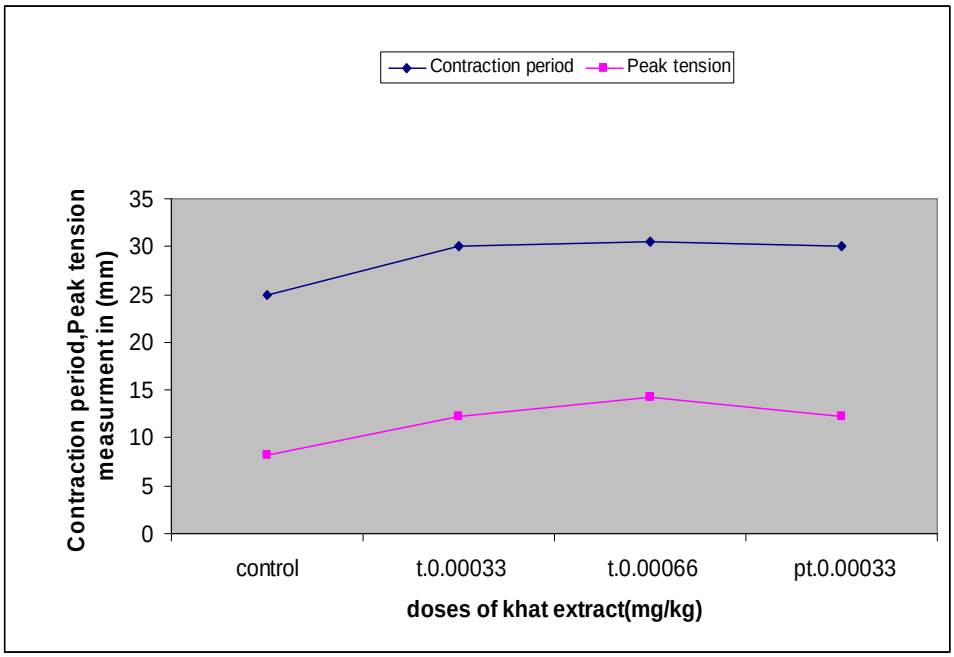
Figure(1): Effect of khat extract on muscle excitability in phases Of contraction period and latent period in comparison with untreated muscle. Measurement in mm.



l.p.: Latent Period. t. : Treatment dose (mg/kg)

c.p.: Contraction Period. Pt. : Pretreatment dose (mg/kg)

Figure (2): Effect of khat extract on contraction period and peak tension Phases of the muscle contraction comparison with control untreated muscle (the control one).



Control : Untreated muscles.

t. : Treated muscles by tow doses.

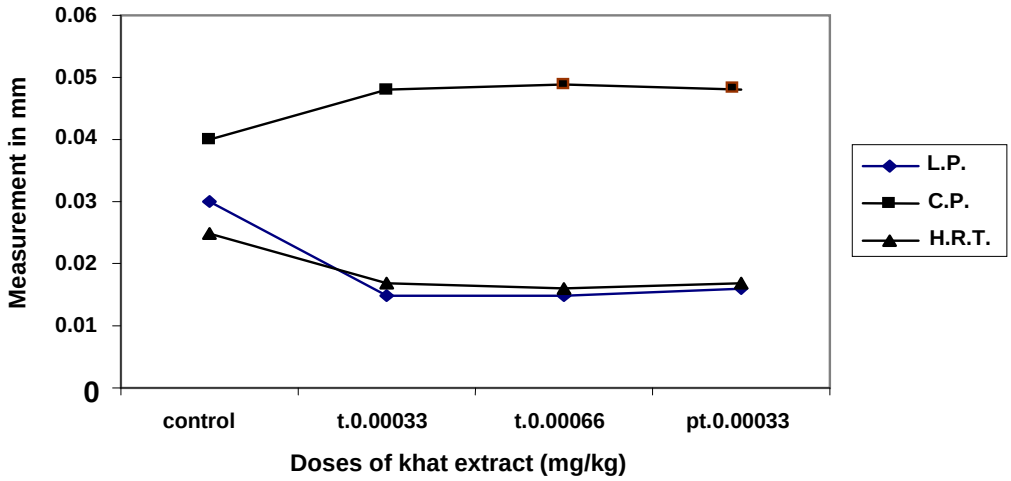
Pt. : Pretreated muscles by lower dose.

Table (2) : Mean value Measurements of muscle contraction's phases in compare between the effects of khat extract on the muscle contraction and the muscle contraction of the control muscle in normal condition. The measurements were recorded in second.

		Untreated muscle	Experimental muscles		
			Treatment	Treatment	Retreatment
			Dose (mg khat extract/ml distill water)		
			23	46	23
			Dose equivalent (mg khat extract/kg body weight)		
No.	Various phases in muscle Contraction (sec.)	Control	0.0057	0.0117	0.0057
1-	Latent Period (L.P.)	13.5mm 3 ✕ 1/100 (0.03)	4mm 1.5 ✕ 1/100 (0.015)	4mm 1.5 ✕ 1/100 (0.015)	4.50mm 1.6 ✕ 1/100 (0.016)

No.	Various phases in muscle Contraction (sec.)	Untreated muscle	Experimental muscles		
			Treatment	Treatment	Retreatment
			Dose (mg khat extract/ml distill water)		
			23	46	23
			Dose equivalent (mg khat extract/kg body weight)		
		Control	0.0057	0.0117	0.0057
2-	Contraction Period (C.P.)	25mm 4 ✕ 1/100 (0.04)	30mm 4.8 ✕ 1/100 (0.048)	30.50mm 4.8 ✕ 1/100 (0.049)	30mm 4.8 ✕ 1/100 (0.048)
3-	Relaxation Period (R.P.)	26.5mm 4.8 ✕ 1/100 (0.05)	19.5mm 4 ✕ 1/100 (0.04)	19.5mm 3.75 ✕ 1/100 (0.038)	19.5mm 4 ✕ 1/100 (0.04)
4-	Half Relaxation Period (H.R.P.)	11.75mm 2.5 ✕ 1/100 (0.025)	7.9mm 1.7 ✕ 1/100 (0.017)	7.5mm 1.6 ✕ 1/100 (0.016)	8mm 1.7 ✕ 1/100 (0.017)
5-	Peak Tension (P.T.) or Maximum recording height of the contraction(Mhc)	8.25	12.75	14.25	12.25

Figure(3): The effect of khat extract on muscle contraction phases In compare with normal one .



L.P.: Latent Period. C.P.: Contraction Period.

H.R.T.: Half Relaxation Time.

Discussion

The simple muscle curve, as obtained with a frog's gastrocnemius has a total duration fore four phases of about 0.1 sec.

The response happened after khat treatment showed the changes in the tension output, contraction time and half relaxation time in compare with untreated control muscle. Whereas the latent period shortened (0.015 second) with the relaxation time (0.012 second).The decrease in the latent period due to the effect of khat which caused: 1)Decrease in the viscosity of the muscle.2)Hasting up of enzymatic and chemical change prior to the mechanical events.(Chatterjee,1994).3) Speeding up of conduction velocity of the nerve impulses (in case the effect of khat extract on excitability was when electrical stimuli applied through the nerve to the muscle). (Thabet,2008).While the decrease in the relaxation time referable to the connection of transversal bridge with presence of Mg^{2+}- ATP that untied the connection of transversal bridge with action filament in addition to the reduction of Ca^{2+} abundance. Accordingly to the increment in contraction period (20ofmm) and the height effect intensity (48.5mm) in retreatment muscle by khat leaves extract in compare with that untreated control muscle because the muscle contracts were at a faster velocity, thus pulled up the lever to a higher record of amplitude (Chai,1995).

These results indicated khat leaves that chewing by Yemenies had highly stimulant effect on muscles as an excitant type of drug caused liberation of enzymatic and chemical energy during contraction process which converted into mechanical energy, where in the terminal storage deposited for Ca^{2+} in cistemae stimulated by AP to release enough Ca^{2+} to bind with troponin in sarcomere to give the initial contraction. The thermo-energy that produced through the ATP hydrolyzed by myosin ATP-ase storage and ADP that were still attached to the myosin molecule interacted with actin to form actomyosin complex in presence of ATP-ase produced the movement to the

muscle. (Allojy, 2002and Hamid, 2004).

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الخلاصـــة

تأثير مستخلص أوراق نبات القات (Catha edulis) على إستثارة العضلة السمانية للضفدع

يتعاطى اليمنيون اوراق شجرة القات بصورة شائعة وفق اعتقاد سائد بان مضغ اوراق القات ينشط الجانب الذهني ويزيد من القدرة على العمل لفترة طويلة .
تمحورت معظم الدراسات حول الاثار السلبية لعادة تعاطي القات التي تقود الي حدوث مشكلات اجتماعية ـ اقتصادية والمخاطر التي تهدد صحة الافراد.
اقيمت هذه الدراسة علي النسيج المستثار المعزول للعضلة السلمانية لباطن ساق العلجوم وذلك بعد تحديد الجرعة المعتمدة لتاثير مستخلص اوراق القات علي العضلة واثبتت التاثير التنبيهي الواضح لمستخلص اوراق القات على الانقباض العضلي والذي كان من السهولة اختباره عند مرور نبضات كهربائية كانت اعطيت خلال العصب او خلال العضلة مباشرة . فالنسيج المعالج بالقات قد اظهرزيادة معلومة مميزة في زمن الانقباض للعضلة السمانية (الوركية) كما وقد احدثت تلك المعالجة انقاصا لكل من فترتي زمن الارتخاء ونصف زمن الرتخاء، أما اعادة المعالجة بمستخلص اوراق القات كان قد سبب تغيرات في ناتج التوتر العضلي ، حيث زادت من ارتفاع تاثير سعة النبضة مع اطالة زمن الانقباض بينما حدث نقصان لفترة الكمون وانقاص ضئيل لنصف زمن الارتخاء بالمقارنة بالعضلة غير المعالجة بمستخلص اوراق القات .
كشفت نتائج هذه الدراسة أن للقات تأثيرات تنبيهية عالية على العضلات حيث يسبب تحرر للطاقة الحرارية- الإنزيمية والطاقة الكيميائية خلال فعل الإنقباض للعضلة والتي تتحول الى طاقة ميكانيكية تكشف العضلة فيها عن نفسها كشغل إستثاري تحت تأثير القات ، كما وقد وجد أن التراكيز المختلفة من مستخلص أوراق نبات القات تسبب جميعها زيادة في الفعل الإستثاري لهذا النسيج العضلـــي .