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Common Carotid Artery Hemodynamic and Brain Weight Effects of *Catha edulis* (Khat)

Abebaye Aragaw Limenie[1*], Eyasu Mekonnen Eshetu[2], Daniel Seyifu Melka[3] and Tesfaye Tolessa Dugul[1]

[1]*Department of Physiology, Addis Ababa University, College of Health Sciences, Addis Ababa, Ethiopia*

[2]*Department of Pharmacology, Addis Ababa University, College of Health Sciences, Addis Ababa, Ethiopia*

[3]*Department of Biochemistry, Addis Ababa University, College of Health Sciences, Addis Ababa, Ethiopia*

[*]**Corresponding author**: Abebaye Aragaw Limenie, Department of Physiology, Addis Ababa University, College of Health Sciences, Addis Ababa, Ethiopia, Tel: +251-118-959055; E-mail: abebaye.aragaw@aau.edu.et

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Abstract

Background: Chewing the leaves of Khat is a social habit in East African countries including Ethiopia. Its health and the socio-economic burden is becoming a severe problem in Ethiopia. Its effects on Doppler velocities in common carotid arteries have not been studied. The present study was, therefore, designed to evaluate its hemodynamic effects on these arteries in wild- type male white albino rats.

Materials and methods: A total of 42 adult (7-8 weeks) wild-type male white albino rats weighing between 213 and 229 g were used in this study. The rats were received Khat extract sub chronically (kesc, 100 mg/kg, 200 mg/kg and 300 mg/kg b.w.), Khat juice (khJ, 2.5 ml/kg), ascorbic acid (AA, 200 mg/kg), 2% tween 80 in distilled water (T80W-v/v) and Khat extract sub acutely (kesa) 300 mg/kg. The blood flow velocities and indices were measured using an ultrasonic Doppler flow meter. The Doppler waveforms were measured three times and the average values were taken for analysis using SPSS version 21.0 and Microsoft Excel.

Results: The SysV of common carotid artery was significantly reduced in rats received the middle ($p<0.01$) and higher ($p<0.01$) doses of Khat extract sub chronically compared with rats received vehicle. However, the DiaV has significantly reduced in rats receiving a higher dose of extract compared with the vehicle ($p<0.05$) and ascorbic acid ($p<0.001$). The SysV and DiaV in rats received the lower dose of Khat extract, Khat juice, AA sub chronically and the higher dose of Khat extract sub acutely were not affected ($p>0.05$). The RI ($p<0.001$) and the PI ($p<0.01$) of this artery was significantly higher in rats received a higher dose of extract compared with the rats received the lower dose. The size (in g) of the brain at a higher dose ($p<0.001$) of extract administered sub chronically was significantly reduced compared with rats received the vehicle.

Conclusions: Khat affected the common carotid artery Doppler velocities and indices particular at the higher doses and prolonged administration. The organ bath *in vitro* effects of Khat on these arteries should be investigated in the future.

Keywords: Doppler sonography; Khat; Common carotid artery; Doppler flow velocities and indices

Introduction

Substance abuse is a major health concern and is becoming a public health issue [1]. The cognitive functions the brain is affected by substance induced vascular abnormalities and related organ function problems [2,3]. Substance induced cerebrovascular [4] maladaptation alters cognitive and behavioral responses.

A study indicated that blood flow into brain was reduced in subjects with cognitive problems [5,6] and a stroke is common among amphetamine and amphetamine like-stimulant abusers [7,8]. One of the substances reported to have stimulatory response related to amphetamines and widely consumed in east African countries including Ethiopia is *Catha edulis* Forsk, commonly called Khat [9,10]. Its health and socio-economic burdens are becoming public problems [10].

Human and animal studies indicated that Khat alters behavioral [11-14], biochemical [15], structural [16] and cardiovascular responses [17,18]. Oral administration of Khat extract or cathinone induced changes on cardiac rate, contractility and blood pressure (BP) [19-23] and BP changes influenced cognition [23,24]. Impairments in the cerebral autoregulation are associated with cerebral edema, hemorrhage, stroke and cognitive impairments [25,26].

However, no studies have been conducted on cerebrovascular hemodynamic effects of Khat extract and juice. The size of organs affected by changes on blood flow and influenced cognitive and behavioral functions [27-30]. Toxic action of Khat on brain through its effect on size of these organs has not been studied. The aim of this study was, therefore, to measure common carotid arteries hemodynamic effects of Khat extract and juice and organ to body weight ratio in rat model.

Materials and Methods

Chemicals

Diethyl ether, chloroform (Siga-Aldrich, Germany), Tween 80, ascorbic acid (Vitamin C) and 70% ethanol were purchased from local suppliers in Addis Ababa, Ethiopia.

Plant materials collection

Bundles of fresh Khat leaves (9 kg) were purchased and collected from Awe day. The plant specimen was identified and voucher number (October 16, 2018, AA002) was given by Addis Ababa University national herbarium of Ethiopia. The specimens were kept at the national herbarium.

Plant material extraction

After the edible parts of the leaves were separated and washed with tap water, the leaves were freeze dried at -20°C [21,31] for 2 days and crushed using mortar and pistil. Two hundred g of freeze dried crushed leaves were placed into a conical flask wrapped with aluminum foil [32]. A total of 400 ml organic solvents i.e. 300 ml diethyl ether and 100 ml chloroform (3:1v/v ratio) were added into the flask. The mixture was shaken under dark condition for 48 hours using a rotary shaker (New Brunswick Scientific Co, USA) at 120 rpm and 20°C. It was then filtered initially using cotton gauze followed by grade I Whatman filter paper (Cat No 1001 150). The organic solvents were then removed through evaporation using Rota-vapor under controlled temperature of 36°C, rotation of 120 rev/minute and 240 Pascal negative pressure. The water in the extract was removed through lyophilization and the dry residue was weighed using analytical balance and stored in a desiccator till used. The Khat juice (khJ) was prepared from 12 g/kg body weight (b.w.) of fresh leaves using 2% tween 80 in distilled water (v/v). The fresh leaves with the tween 80 in distilled water (T80W) were crushed using blender machine. The juice was then squeezed and filtered using the gauze and grade I Whatman filter paper (Cat No 1001 150). The amount of juice extracted from Khat leaves for each rat was determined from the total weight of each rat and selected leaves weight (12 g/kg b.w.). The amount of T80W used to extract the given weight of the leaves was determined based on the total weight of each rat and standard vehicle volume (2.5 ml/kg b.w.).

Animal preparation

A total of 42 adult wild-type male white albino rats aged between 7 and 8 weeks obtained from same breeding series weighing between 213 and 229 g were used. The rats were purchased from Laboratory Animal Breeding Section of Ethiopian public health institution. Three rats per plastic cages under natural light and dark (12:12 hours) cycles at room temperature were housed. Rats were weighed twice a week to ensure appropriate dosing based on body weight changes. Water and standard pellet diet were available ad libitum throughout the experimental period. Rats were weighed twice a week to ensure appropriate dosing based on body weight changes. Each rat was handled in accordance with the guidelines for animal research as detailed in the NAP guidelines for the Care and Use of Laboratory Animals [33] and were approved by ethical committee in Addis Ababa University.

Grouping and dosing

The rats were randomly assigned into seven groups (n=6 /group) received T80W, Khat extract subchronically (kesc) (kesc 100 mg/kg, kesc 200 mg/kg and kesc 300 mg/kg), ascorbic acid (AA 200 mg/kg) and Khat juice (khJ 2.5 ml/kg) subchronically and Khat extract subacutely (kesa) kesa 300 mg/kg. Subchronic groups of rats were received the test substances for thirteen weeks prior to this experiment. However, the subacute group received the extract only nine days. The T80W (vehicle) and ascorbic acid were taken as negative and positive controls, respectively. The doses for Khat extract were selected based on previous reports [34,35].

Preparation of test substances and volume determination

Fresh solution of extract, AA, khJ and vehicle were prepared every day. The Khat extract was dissolved in T80W. Ascorbic acid was powdered and dissolved in the T80W to make a sock solution of 80 mg/ml. The dose of the extract administered in each rat was calculated from selected doses (100 mg/kg, 200 mg/kg and 300 mg/kg) and the total body weight (b.w.) of each rat. The appropriate standard vehicle volume (2.5 ml/kg b.w.) was used to determine how much volume was used to dissolve the calculated dose of Khat extract and AA. Each rat in its respective group received a single daily oral administration of vehicle, Khat extract, khJ and AA. The final volume for each rat was 1 ml and all substances were administered orally using metal gavage needle.

Measurement of Doppler flow velocities and indices

Doppler flow velocities and indices that measure vascular changes, flow resistance and toxic effects of substances [36-38]were determined in this study and this parameters reflect the systemic hemodynamic changes [39-41].The peak systolic flow velocity (SysV), end diastolic flow velocity (DiaV), mean flow velocity (Mfv), resistivity index (RI=SysV-DiaV/SysV) and pulsatility index (PI=SysV-DiaV v/mean velocity) effects of Khat and ascorbic acid were measured in this study and the measurement was conducted 24 hours after the last test substances administration.

Experimental design

The protocol used by Domínguez et al. [42] and Ostrowska et al. [43] was used with little modifications. Briefly, each rat was exposed to diethyl ether to anesthetize before surgical procedure. Tracheostomy and catheterization were performed for artificial breathing using tracheostomy tube and electric geared motor device (PARVALUX, England). The right and the left common carotid arteries were exposed where the probe (8 MHz) was placed on the surface of the vessels at the angle of 450 between the ultrasound beam and the direction of blood flow. The probe was placed opposite to the direction of the blood flow. The blood vessel Doppler Waveforms were recorded using ultrasonic laser Doppler flow meter (Atys Medical) at pre-set constant Doppler frequency of +250 Hz. The mean value of the hemodynamic data was determined by averaging three Doppler waveforms. The SysV and DiaV were recorded from which Mfv, RI and PI were determined and the measurements were conducted on 24 hours after the test substances were administered.

Organ weight measurement

Following Doppler measurement and scarification of rats, whole brain was removed and weighed using an analytical balance. The percentage of the brain to the body weight (relative weight) was determined.

Statistical analysis

The statistical analyses was done using SPSS version 21.0 and graphs were plotted using Microsoft excel. The values were expressed as mean ± SEM. Differences in mean vascular velocities, indices, brain sizes and organs to the total body weight ratio of all groups of rats were analyzed using one way ANOVA followed by post hoc Tukey's test. Independent t test and bivariate analysis was also used. P value less than 0.05 was considered significant difference between the groups.

Results

In this study, the ultrasonic Doppler values were represented in waveforms (Figure 1).

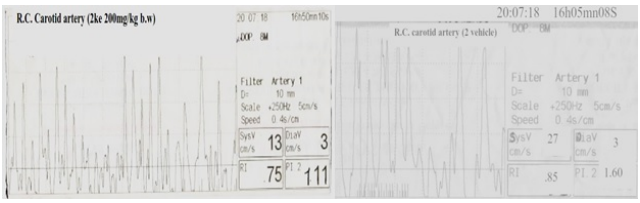

Figure 1: Ultrasonic Doppler waveforms in rat received kesc 200 mg/kg b.w. (right) and vehicle (left) for twelve weeks. 2 represents rat No 2 from each group.

Effects of Khat on Common carotid artery hemodynamics

Peak-systolic and end-diastolic flow velocities (cm/s): The one way ANOVA (F (6,35)=5.25, p<0.01) followed by post hoc Tukey's test indicated that SysV was significantly reduced in rats received kesc 200 mg/kg and kesc 300 mg/kg compared with rats received vehicle (8.58 ± 1.78 *vs.* 18.00 ± 3.51, p<0.05 and 6.73 ± 1.12 *vs.* 18.00 ± 3.51, p<0.01 respectively) as shown in Figure 2. However the DiaV was significantly reduced in rats received kesc 300 mg/kg (p<0.05) compared with vehicle. The SysV in this artery in rats received kesc 300 mg/kg was significantly reduced compared with rats received kesa 300 mg/kg (p<0.01). The DiaV of common carotid artery was significantly increased in rats received AA 200 mg/kg compared with rats received kesc 200 mg/kg (p<0.01), kesc 300 mg/kg (p<0.01) and khJ 2.5 ml/kg (p<0.05). The DiaV in this artery among rats received kesc 300 mg/kg was significantly reduced compared with rats received kesc 100 mg/kg (p<0.05).

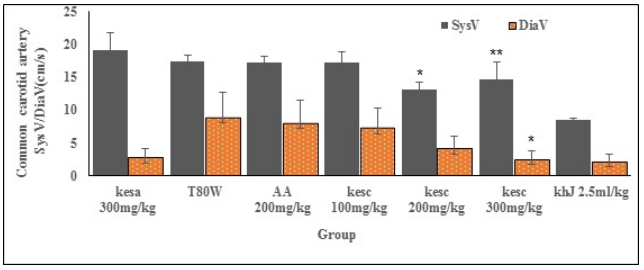

Figure 2: Effects of Khat on common carotid artery SysV and DiaV. Rats (n=6 in each group) were received kesa 300 mg/kg, T80W, AA 200 mg/kg, kesc 100 mg/kg, kesc 200 mg/kg and kesc 300 mg/kg and khJ 2.5 ml/kg. Each bar represents mean ± SEM of these velocities and **p<0.01 and *p<0.05 when each group was compared with rats received T80W. kesa: Khat extract subacute, kesc: Khat extract subchronic and khJ: Khat juice.

Common carotid artery mean flow velocity (cm/s): There was significant difference in common carotid artery Mfv (F (6,35)=5.24, p<0.01) among groups. Rats received kesc 300 mg/kg had significantly reduced Mfv compared with rats received vehicle (p<0.01), kesa 300 mg/kg (p<0.01) and ascorbic acid (p<0.01) as shown in Table 1 and Figure 3.

Common carotid artery resistivity and pulsatility indices: There was significant difference in the carotid artery RI (F (6, 35)=6.38, p<0.001) and PI (F (6, 35)=4.58, p<0.01) among all groups. However, the post hoc Tukey's test was not revealed significant difference in the RI and PI when each group of rats was compared with rats received vehicle (Table 1). The RI (0.98 ± 0.09 vs. 0.57 ± 0.04, p<0.001) and the PI (1.38 ± 0.12 vs. 0.57 ± 0.04, p<0.01) in rats received kesc 300 mg/kg was significantly higher compared with rats received ascorbic acid and this RI, but not the PI (R=-0.125, PP>0.05), in this group of rats was significantly associated with the SysV (R=-0.99, p<0.001), as shown in Figure 4. The carotid artery RI (0.98 ± 0.09 vs. 0.59 ± 0.01) and PI (1.32 ± 0.12 vs. 0.82 ± 0.04, p<0.01) was significantly increased among rats received kesc 300 mg/kg compared with the rats received kesc 100 mg/kg and the RI in this group was not significantly associated with the SysV (R=-0.076, p>0.05). However, the RI (0.76 ± 0.04 vs. 0.78 ± 0.05, p>0.05; 0.76 ± 0.04 vs. 0.57 ± 0.04, p>0.05 and 0.76 ± 0.04 vs. 0.59 ± 0.01) and the PI (1.23 ± 0.09 vs. 0.96 ± 1.2; 1.23 ± 0.09 vs. 0.82 ± 0.08, p>0.05 and 1.23 ± 0.09 vs. 0.82 ± 0.04, p>0.05) in rats treated with kesa 300 mg/kg was not significantly increased compared with the rats received vehicle, ascorbic acid and the kesc 100 mg/kg.

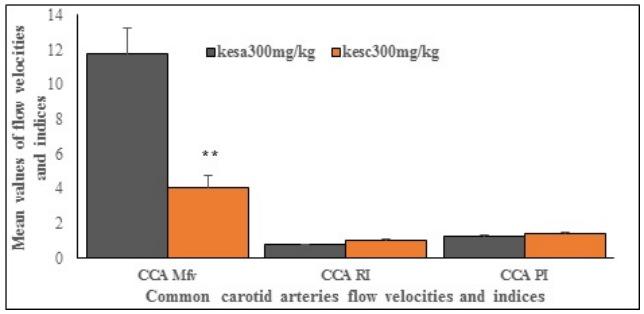

Figure 3: Common carotid arteries velocities and indices in rats (n=6 in each group) received Khat extract kesa 300 mg/kg and kesc 300 mg/kg. Each bar represents mean ± SEM of Doppler velocities and indices. **p<0.01 when common carotid artery of rats received kesa 300 mg/kg was compared with rats received kesc 300 mg/kg. CA: common carotid artery, Mfv: mean flow velocity, RI: resistivity index, PI: pulsatility index.

Parameter M ± SEM	Groups of rats received vehicle, AA, Khat extract(ke) and Khat juice(khJ)						
	Vehicle T80W	AA 200 mg/kg	Kesa 300 mg/kg	Kesc 100 mg/kg	Kesc 200 mg/kg	Kesc 300 mg/kg	khJ (2.5 ml/k)
CCAMfv	10.50 ± 1.88	10.52 ± 1.18	11.75 ± 1.52	7.72 ± 1.07	5.37 ± 1.09	4.05** ± 0.74	7.35 ± 0.95
CCARI	0.78 ± 0.05	0.57 ± 0.04	0.76 ± 0.04	0.59 ± 0.01	0.77 ± 0.07	0.98 ± 0.09	0.73 ± 0.03
CCAPI	0.96 ± 0.12	0.82 ± 0.08	1.23 ± 0.09	0.82 ± 0.04	1.14 ± 0.12	1.38 ± 0.12	1.17 ± 0.09

Each point represents the mean ± SEM of common carotid artery mean flow velocity (CCAMfv), resistivity index (CCARI), pulsatility index (CCAPI) in rats (n=6 in each group) received T80W, ascorbic acid (AA), Khat extract sub acutely (kesa) and Khat extract sub chronically (kesc) and Khat juice (khJ) sub chronically. ***p<0.001, **p<0.01 and *p<0.05 when each group of rats was compared with rats received T80W.

Table 1: Effects of Khat on common carotid arteries ultrasonic Doppler parameters.

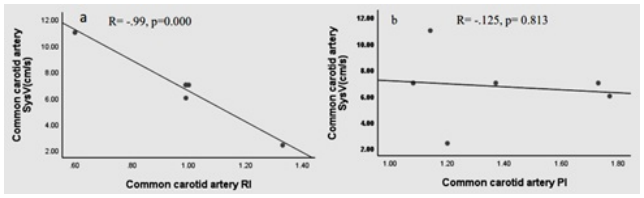

Figure 4: Correlation between the common carotid artery SysV and RI (a) and PI (b) among rats received kesc 300 mg/kg. R: correlation coefficient.

Effects of Khat on the body weight and organ to body weight ratio

Total body weight: Although there was no significant difference in total body weight (b.w.) at the beginning of the study (F (5, 30)=0.204, p>0.05), difference was observed (F(3, 30)=3.86, p<0.01) at the end of the study. However, the post hoc analysis indicated that significant difference in the total b.w. measured at the end of the study was not observed in rats received ascorbic acid, kesc 100 mg/kg, kesc 200 mg/kg, kesc 300 mg/kg and khJ (2.5 ml) (p>0.05) as shown in Table 2. The total body weight in rats received Khat extract and juice sub chronically was not significantly associated with the common carotid artery RI(R=-0.384, p>0.05 and R=-0.211, p>0.05) and PI (R=-0.142, p>0.05 and R=-0.127, p>0.05, respectively). The common carotid artery in rats received kesc 300 mg/kg, was not significantly correlated with the total body weight (R=-0.007, p>0.05) as shown in Figure 5.

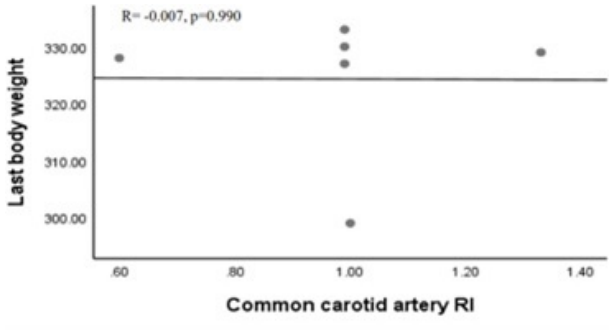

Figure 5: Correlation between common carotid artery RI and body weight at the end of experiment in rats received kesc 300 mg/kg. R: correlation coefficient, RI: Resistivity Index Brain and its relative weight.

Significant difference in brain weight was observed among groups ($F(5, 30)=16.19$, $p<0.01$). The brain weight in rats received the kesc 300 mg/kg was significantly reduced compared with rats received vehicle ($p<0.001$), AA ($p<0.001$), and kesc 100 mg/kg ($p<0.001$). Its relative weight was also significantly reduced in rats received kesc 200 mg/kg ($p<0.05$) and the kesc 300 mg/kg ($p<0.01$) compared with the vehicle. However, there was no significant correlation between weight of the brain and SysV in these group of rats ($R=0.115$, $p>0.05$ and $R=-0.653$, $p>0.05$, respectively).

Parameters M ± SEM	Groups of rats received vehicle, AA, Khat extract and juice					
	vehicle	AA	Ke100 mg/kg	Ke200 mg/kg	Ke300 mg/kg	khJ (2.5 ml/kg)
initial b.w. (g)	221.00 ± 2.05	222.50 ± 1.80	222.83 ± 1.64	221.50 ± 1.69	222.50 ± 1.43	221.00 ± 2.18
last b.w. (g)	333.00 ± 1.18	339.67 ± 3.44	334.67 ± 1.31	336.17 ± 1.45	324.33 ± 5.14	337.16 ± 0.79
BW (g)	2.10 ± 0.01	2.09 ± 0.04	2.05 ± 0.02	2.02 ± 0.03	1.75*** ± 0.04	2.02 ± 0.04
BbR	0.63 ± 0.00	0.62 ± 0.01	0.61 ± 0.00	0.59* ± 0.01	0.54*** ± 0.01	0.60 ± 0.01

Each point represents mean ± SEM of total body weight (b.w.) at the beginning of the study, at the end of the study, brain weight (BW) in gram (g), brain to body weight ratio (BbR) in rats (n=6 in each group) received vehicle, ascorbic acid (AA), Khat extract (Ke) and juice (khJ) sub chronically. ***p<0.001, **p<0.01 and *p<0.05 when each group of rats was compared with the vehicle.

Table 2: Effects of Khat on total body weight, brain and its relative weight in rats.

Discussion

Common carotid artery flow velocities (cm/s)

In this study, common carotid artery SysV was significantly reduced in rats received the lower and the higher doses of Khat extract administered subchronically. However, the DiaV in this artery was significantly reduced among rats received the higher dose of the extract compared with rats received vehicle and lower dose of extract The SysV of this artery was significantly reduced among rats received the higher dose of extract administered subchronically compared with rats received the same dose subacutely indicating that Khat extract administered subacutelly couldn't reduce this SysV. This finding was in agreement with the previous study [17] that revealed the SysV was significantly increased following acute Khat chewing. The mean flow velocity was significantly reduced among rats received the higher dose of Khat extract subchronically compared with rats received hat extract subacutely, the lower dose of Khat extract subchronically, vehicle and ascorbic acid. The findings in this study showed that the effects of Khat extract on the carotid artery SysV and DiaV was dose and time dependent. The study conducted by Wang et al. [44] showed that the acute and low dose of amphetamine, amine with cathinone in the Khat like effect, and cocaine produced mild vasodilatation and increased flow and velocity in basilar artery of rabbit. However, higher dose and prolonged administration of amphetamine like stimulants reduced blood flow into the brain and amphetamine reduced the flow among patients with attention deficit hyperactive disorder pre-exposed to stimulants [45].

Common carotid artery flow indices

The common carotid artery RI and PI were significantly increased among rats received the higher dose of extract subchronically compared with rats received ascorbic acid and the lower dose of the extract, but not with vehicle received rats. The higher RI of this artery, but not the PI, was inversely affected the flow velocity during systolic phase of the cardiac cycle. The RI and the PI of rats received the extract at a higher dose subacutely were not significantly reduced compared with rats received vehicle and ascorbic acid. However, previous study indicated that the common carotid artery RI was significantly reduced in humans following Khat leaves chewing [17]. The dissimilarity between these findings could be attributed to the time of the Doppler measurement in that the Doppler measurement was made 24 hours after the last administration of Khat in our study but it was immediately following Khat chewing in the previous study. Another study also indicated that flow of blood into brain was increased 5 minutes after administration of amphetamine and reduced 30 minutes later [46]. Amphetamine at a small dose and acute administration dilated cerebral artery and reduced resistance and RI. The SysV of rats received the lower dose of the extract subchronically was not affected

by RI indicating that there could be factors other than RI that affect the SysV. A previous study indicated that RI and cerebral blood flow was affected by the pumping strength of the heart [47,48]. In vitro studies were also showed that cardiac contractility and rate were reduced [21,49], while aortic contractility was increased [50] by Khat extract. Thus, the effect of Khat on the Doppler RI could by through its effect on heart or blood vessels. The PI of the common carotid artery was significantly higher in rats received the higher dose compared with the lower dose of Khat extract. This result showed that low dose of the extract reduced the variability of blood flow velocity which is observed when the blood vessels are dilated and resistance is reduced. A study indicated that calcium channel blockers dilate the blood vessels and reduced the PI of blood vessels [51].

Effects of Khat on body and organ weight

Total body weight: Previous study revealed that Doppler values were affected by the total body weight and body mass index [52]. In this study, no significant differences in the total body weight were observed among rats received Khat compared with rats received vehicle. However, previous study indicated that the total body weight of rats received higher doses of Khat extract (1000 and 2000 mg/kg for six weeks) was significantly reduced [16]. The discrepancy in these results could be attributed to the differences in the doses and solvent used. The maximum dose we used in this study was 300 mg/kg and the solvent used to extract Khat were diethyl ether and chloroform while it was ethanol in the previous study. Other review also showed that Khat reduced the total body weight of animal [53]. However, the total body weight of rats received Khat at a higher dose subchronically was significantly reduced compared with rats received ascorbic acid and the total body weight of rats received Khat had no effects on the common carotid artery RI and PI. This indicated that altered Doppler RI and PI observed could be attributed to factors other than the body weight of the rats.

Brain to body weight ratio: The higher dose of the Khat extract reduced the weight of the brain significantly, but its relative weight was reduced by both the middle and the higher dose of extract administered sub chronically. These results indicated that the effects of the Khat extract on the size of the brain were dose dependent. However in the study conducted by Isaac et al. [16], the size of the brain and its weight to the body weight ratio was reduced at a higher dose of extract, 2000 mg/kg. The reason for the reduction of the brain size and its weight to the body weight ratio at a dose of 300 mg/kg in our study could be attributed to the duration of administration, it was for twelve weeks in our study and was six weeks in the previous study. These results showed that Khat has toxic effect on brain and previous study indicated that sizes of organs were affected by the toxicity effects of substances [54]. Generally, the reduction in the size of the brain in this study could be attributed to oxidative stress, lipid peroxidation [55,56], vasoconstriction, congestion, infarction, thermo genic and energy expenditure [57,58] and other adverse effects of the Khat.

Conclusion

Previous studies reported that Khat contributed for oxidative stress and lipid peroxidation. Amphetamine and related compounds also increased the body temperature and energy expenditure that could affects the organ weight. In conclusion, Khat affected common carotid arteries Doppler velocities and indices particular at a higher dose and prolonged administration. The organ bath *in vitro* effects of Khat on these arteries should be studied in the future.

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