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EFFECT OF KHAT-HABITUATION ON GABA LEVELS IN BRAIN

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ABSTRACT

Khat (Catha edulis forsk; family: Celastraceae) is a plant habitually chewed by several millions of people in Yemen and southern east areas of Africa for its pleasant stimulant effect on physical activity, consciousness, motor and mental functions as well as its anti-fatigue action. This study was aimed to investigate the implication of chronic khat-habituation on levels of the main inhibitory neurotransmitter, GABA, in relevant areas of brain namely cerebral cortex, midbrain and hindbrain in an animal model. Eighteen mice (20-30gm) were used in this study; they were grouped into control, Khat-habituated, and khat-withdrawal. Khat-habituation was induced by providing khat (2g/kg) with food for two months. Khat-withdrawal was induced by cessation of khat-containing food for 14 days. The GABA levels in brain areas were determined quantitatively using chromatographic and calorimetric method. Khat-habituated animals showed significant decrease in GABA contents in tested brain areas. Khat-withdrawal for 14 days reversed effects caused normalization for GABA levels in brain. Chronic khat-habituation has transient deleterious effect on the levels of GABA in the brain which may reinforce the development and spread of epileptic discharges, this effect may be reversed after its cessation.

Key words: *Khat (Catha edulis), GABA, epilepsy.*

INTRODUCTION:

Khat (Catha edulis forskal, family: Celastraceae) is a plant grown in Yemen and on the eastern coast of Africa [1, 2]. The leaves this plant are habitually chewed by several millions of people in Yemen and southern east areas of Africa for its pleasant stimulant effect on physical activity, consciousness, motor and mental functions as well as its anti-fatigue action. The principal active constituents of khat are cathinone and cathine, which have sympathomimetic actions [3].

Gamma-amino-butyric acid (GABA) represent the main inhibitory neurotransmitter in the brain. An imbalance between glutamate and GABA neurotransmitter systems can lead to hyper-excitability which plays a role in epileptogenesis [4]. In most situations GABA is found in short interneurons, the only long GABAergic tracts being those running to cerebellum and striatum namely the major output of cerebral cortex, olfactory bulb, hippocampus

and lateral septal neurons. GABA also mediates inhibition within cerebral cortex and between caudate nucleus and substantia nigra. The latter pathway may mediate decrease in dopamine release[5].

The present study was aimed to investigate the implication of chronic khat-habituation on levels of the main inhibitory neurotransmitter, GABA, in relevant areas of brain namely cerebral cortex, midbrain and hindbrain in an animal model. This can explore the possible effect of chronic khat-habituation on development of epileptic seizures.

METHODS:

This study was carried out in Pharmacology and Therapeutics Laboratory of Faculty of Medicine and Health Sciences, Sana'a University.

Animals: Eighteen adult male mice (Mus musculus) weighing 20-30 gm obtained from the animal house of Faculty of Science of Sana'a University were used for this study. Mice were housed individually in cages, maintained under standard conditions (12 hours light: 12 hours dark cycle; 25 $\pm 3^0$C). The experiments were performed based on animal ethics guidelines of University Animals Ethics Committee. The animals were randomly divided into 3 groups consisting of 6 mice in each. The animals were randomly divided into 3 groups consisting of 6 mice in each. Group 1 were normal animals served as "control group". Group 2 "khat-habituation group" were animals received khat in a dose of 2 g/kg body weight for two months. Group 3 "khat withdrawal group" were animals received khat in a dose of 2 g/kg body weight for two months then withdrawn from khat habituation for two weeks.

Khat-habituation: khat-habituation was induced by providing khat in a dose of 2g/kg with food after an overnight starvation. After one week of forced khat-feeding, the tested animals were provided with both khat-containing and non khat-containing foods. The majority of the tested animals preferred khat-containing food, this model is similar in some aspects the classical self administration model used in testing drug habituation [6, 7]. Then, animals showing khat-habituation evidenced by marked preference of khat-containing food were continued to feed khat-containing food for two months, other animals were excluded from the study due to resistance to khat-habituation.

Determination of GABA levels in certain brain areas in mice: After the end of experimental period, all animals were rapidly sacrificed by decapitation. The heads of mice were isolated and frozen in less than one minute using a mixture of ice, granular sodium chloride and acetone to avoid postmortem increase in GABA to the free form. After freezing and hardening, the brains were isolated and dissected for specific brain areas namely midbrain, hindbrain and cerebral cortex. GABA levels were quantitatively determined using a chromatographic and calorimetric method as described by *Condsen et al*[8].

Statistical analysis: Results are presented as mean ± S.E.M. and statistical differences between groups and their respective control for evaluation of experimental results was determined by Student's Unpaired Sample t-test using SPSS version 15.0. The level of significance was set at $p<0.05$.

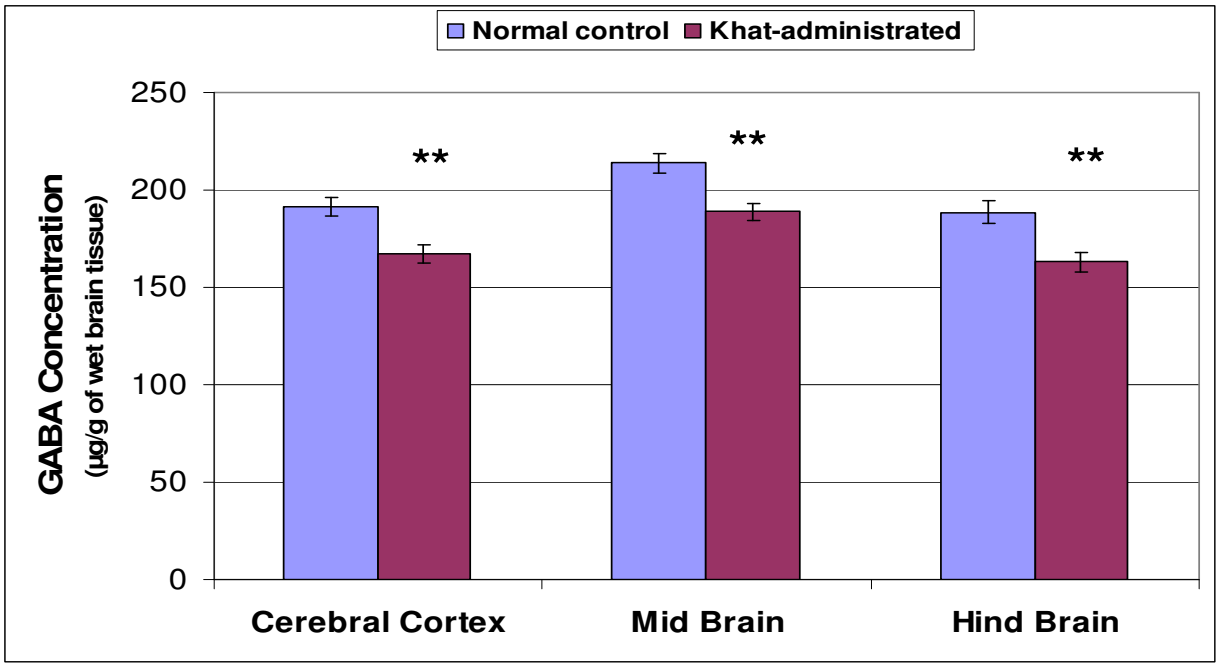
RESULTS:

Effect on GABA levels in relevant areas of the brain: Khat-habituation (group 2) was associated with significant decrease of GABA levels in cerebral cortex, mid brain and hind brain compared with the normal control mice (Table 1, Figure 1).

Table (1): Effect of Khat-habituation (2 g/kg PO for two months) on GABA levels (µg/g of wet brain tissue) in different brain areas mice (Mean±SE, N=6):

Group	**Concentration of GABA** (µg/g of wet brain tissue)		
	Cerebral cortex	**Mid brain**	**Hind brain**
Normal control group (g 1)	**191.37** ± 4.4	**213.7** ± 5.15	**188.6** ± 6.05
Khat administrated group (g 2)	**167.5** ± 4.61	**188.72** ± 4.48	**163** ± 5.13
Percentage of change	- 12.5 **	- 11.7 **	- 13.6 **

**: significant at $P \leq 0.01$



**: significant at $P \leq 0.01$

Figure (1): Effect of Khat-habituation (2 g/kg PO for two months) on GABA levels (µg/g of wet brain tissue) in different brain areas mice (Mean±SE, N=6).

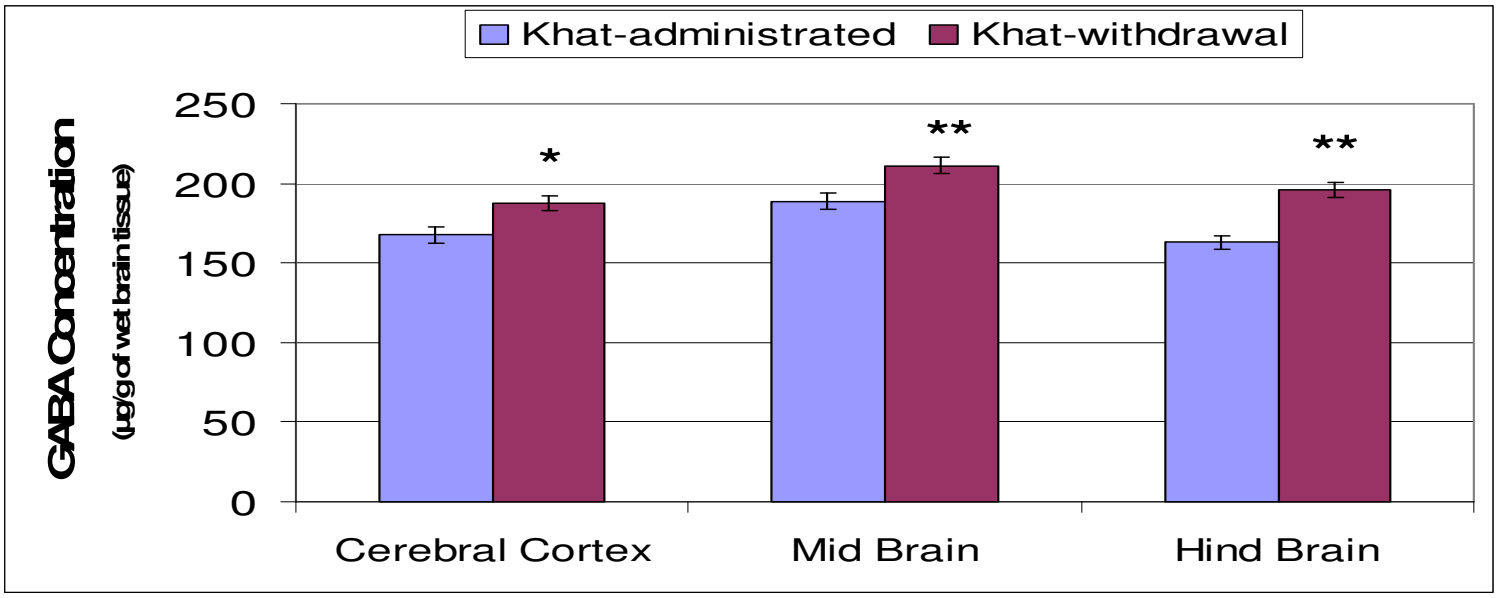
Khat-withdrawal was associated with significant increase in GABA levels in cerebral cortex, mid brain and hind brain compared with khat-habituation mice "group 2" (Table 2, Figure 2).

Table (2): Effect of khat-withdrawal after khat administration on GABA levels (µg/g of wet brain tissue) in different brain areas compared to khat-habituated mice (Mean±SE, N=6):

Group	**Concentration of GABA** (µg/g of wet brain tissue)		
	Cerebral cortex	**Mid brain**	**Hind brain**
Khat-administrated group (g 2)	**167.5** ± 4.61	**188.72** ± 4.48	**163** ± 5.13
khat-withdrawn group (g 3)	**187.43** ± 4.91	**211.1** ± 5.19	**195.9** ± 4.27
Percentage of change	+ 11.9*	+ 11.9**	+ 20.2 **

**: significant at P≤ 0.01

*: significant at P≤ 0.05



**: significant at P ≤ 0.01

Figure (2): Effect of khat-withdrawal after khat administration on GABA levels (µg/g of wet brain tissue) in different brain areas compared to khat-habituated mice (Mean±SE, N=6).

There was no significant difference between khat-withdrawal and normal control mice These values were insignificant compared with normal control mice "group 1" (table 3 and figure 3). This means that khat-induced changes in GABA levels were normalized.

Table (3): Effect of khat-withdrawal after khat habituation on GABA levels (µg/g of wet brain tissue) in different brain areas of khat-withdrawn mice compared to adult normal control mice (Mean±SE, N=6):

Group	**Concentration of GABA** (µg/g of wet brain tissue)		
	Cerebral cortex	**Mid brain**	**Hind brain**
Normal control group (g 1)	**191.37** ± 4.41	**213.7** ± 5.15	**188.6** ± 6.05
khat-withdrawn group (g 3)	**187.43** ± 4.91	**211.1** ± 5.19	**195.9** ± 4.27
Percentage of change	**- 2.1**	**- 1.2**	**+ 3.9**

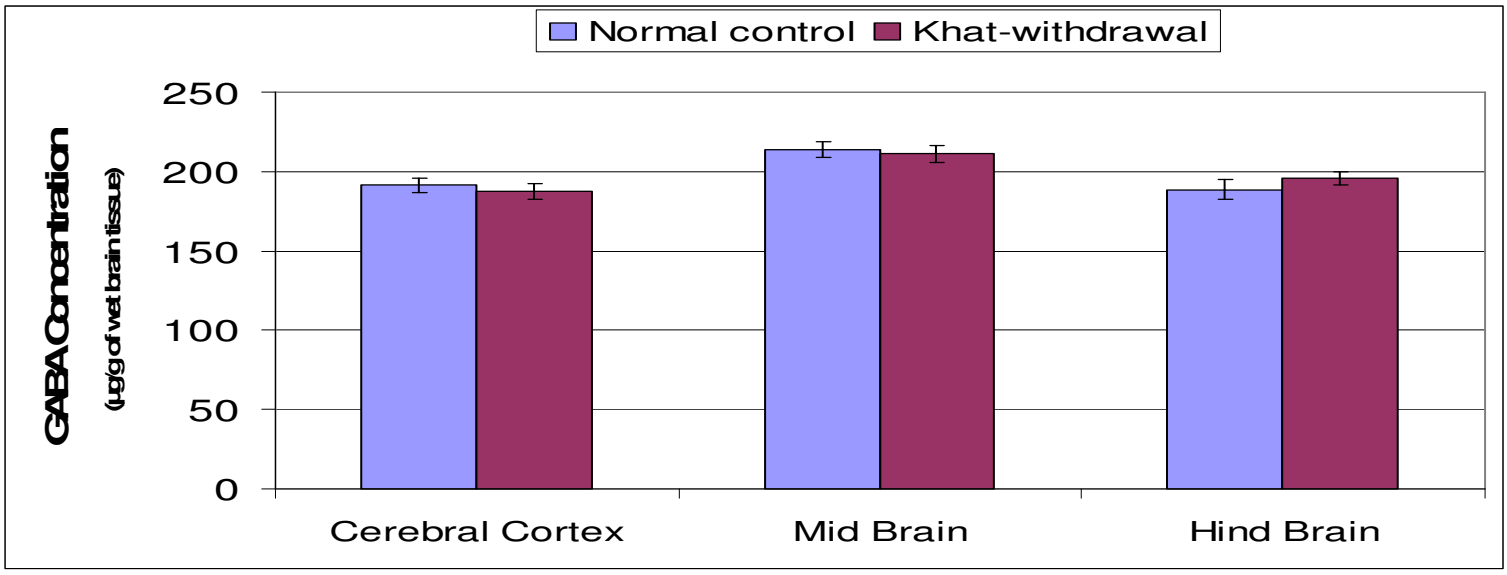


Figure (3): Effect of khat-withdrawal after khat habituation on GABA levels (µg/g of wet brain tissue) in different brain areas of khat-withdrawn mice compared to adult normal control mice (Mean±SE, N=6).

DISCUSSION

The principal active constituents of khat are cathinone and cathine, which have sympathomimetic actions. They facilitate neurotransmission by stimulation of release of catecholamines (mainly dopamine and noradrenaline) stored in the presynaptic nerve terminals. Cathinone is characterized by its greater lipophilicity, to penetrate more easily into central nervous system than cathine, so the peripheral effects induced by khat can be considered to be predominantly due to cathine while the central effects due to cathinone [3].

The reinforcement behavior that encouraged continuous khat consumption (Khat-habituation) in mice in this study may be mood elevation, increase motor activity and resistance to fatigue. This assumption is supported by the preliminary study of Van der Schoot who found that cathinone – one of the active principles of khat – has about half the potency of amphetamine in increasing the spontaneous locomotor activity in mice [9]. Cathine, the other active

principle of khat, has one tenth of potency of amphetamine in increasing the locomotor activity in mice [10]. The stimulant effect of khat was also observed in monkey which showed marked restlessness and resistance to fatigue after self administration of cathinone [11].

In this study the concentration of GABA in brain areas most relevant to epileptic activity namely cerebral cortex, mid brain and hind brain were determined. The normal control animals showed GABA distribution was in consistency with previous studies. It is well correlated with contents of cell bodies in tested brain areas [12]. Khat-habituated animals showed significant decreased concentration of GABA in all tested brain areas. This may be due to depletion of this inhibitory neurotransmitter after excessive prolonged release during long-term khat-consumption, central compensatory response to the sympathomimetic effect of khat. Khat may induce a pressor response due to indirect sympathomimetic action, this stressful condition may initiate a compensatory response to ameliorate such effect through increase GABA release. This assumption is in conformation with the work of Moroni et al[13], who showed that α_1-receptor stimulation increased GABA release in hind brain. Moreover, clonidine which is partial α_2–receptor agonist increased release of GABA. This effect is not mediated through α2–receptors stimulation. Alternatively, such effect may be mediated by stimulation of presynaptic α2-heteroreceptors in the brain which increase GABA release probably through disinhibiting of GABA release inhibitory mechanisms [14]. The released GABA is more amenable for metabolic degradation by glial and neuronal uptake followed by metabolic degradation by GABA transaminase and Succinic semialdehyde dehydrogenase enzymes [12].

Putting all these facts together, one can conclude that prolonged khat consumption increased norepinephrine release through the previously mentioned indirect sympathomimetic effect. This released norepinephrine increases the release of GABA via either direct stimulation of α_1-receptor or stimulation of α_2-receptors which disinhibit GABA release inhibitory mechanisms. The released GABA is more liable for degradation by glial and neuronal uptake. The latter may explain khat mediated decrease in GABA concentration. This effect was completely reversed after khat withdrawal for two weeks.

CONCLUSION:

Chronic khat-habituation in mice resulted in decreasing of GABA levels in the cerebral cortex, mid brain, and hind brain. This may reinforce the development and spread of epileptic discharges, this effect may be reversed by its khat cessation.

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