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The Effect of Acute and Subchronic Administration of Crude Khat Extract (Catha Edulis F.) on Weight in Mice

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Abstract

People widely chew khat, particularly in East Africa, for different purposes but there is no consistent data showing its effect on anorexia and weight. This study was made to add to the existing body of knowledge about khat and its effect on weight. A total of 50 albino mice, 6-8 weeks old, were administered orally with a single daily dose of khat extract for 30 days. The animals were divided into 4 groups. The first group served as controls and was administered with 0.5 ml 3% Tween 80 in water. Group two (K100), three (K200) and four (K300) were administered 100, 200 and 300 mg/kg khat extract, respectively. The effect of crude khat extract on weight was measured and analyzed using One-way ANOVA. The result showed that acute administration of crude khat extract at doses used did have a significant effect on weight on all the three groups (khat administered) ($p< 0.01$) but there was no significant weight difference among the three groups. After the fourth days, this weight decrease, however, was followed by regaining the initial weight and then increasing till the end of the experiment. Crude khat extract at acute and sub-chronic administration have anorexic effect but followed by development of tolerance which may be explained by a complex of Neuro-chemical processes.

Keywords: Catha edulis; Anorexia; Weight; Acute; Subchronic

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1. Introduction

The stimulant leaf khat (*Catha edulis* Forsk) comes from a tree (of the family Celastraceae) which grows in countries bordering the Red Sea, along the east coast of Africa and in west Asia. *Catha edulis* is popularly known as "khat" but is also known as "kat", "qat", "qad", "qaad". "jaad", and "miraa" [1]. People living around the horn of Africa, East Africa and the Middle East [2, 3] have consumed it for centuries. Khat chewing is a widespread habit that has a deep-rooted socio-cultural tradition in these countries causing many socio economic problems [2].

Khat is chewed for recreational purposes and its valued psychostimulant effect is highest when fresh. The aforesaid explains the users' preference for the fresh khat [3]. Fresh khat leaves mainly contain cathinone, a psychostimulant that is similar in structure and pharmacological activity to amphetamine [3, 4, 5]. Due to these similarities, cathinone has been called a 'natural amphetamine' [1, 6]. It is this psychostimulant effect that accounts for the popularity of khat [4].

Many different compounds are found in khat including alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins and minerals. The phenylalkylamines and the cathedulins are the major alkaloids. The cathedulins are based on a poly-hydroxylated sesquiterpene skeleton and are basically polyesters of euonyminol. It has been reported that 62 different cathedulins from fresh khat leaves were characterized [7, 8].

The pharmacologically active constituents of khat are (-)- cathinone and, to a lesser extent, (+)-norpseudoephedrine [7,9]. Cathinone is presumably the main psychoactive component of khat, this explains why fresh leaves are preferred and why khat is wrapped up in banana leaves to preserve freshness [8, 10].

Similar to psychostimulants, khat ingestion produces several central nervous system effects, including increased motor stimulation, euphoria, and a sense of excitement and energy [1]. Khat usage is associated with memory impairment, depression and psychoses [11]. It also results in decreased appetite and increased blood pressure and heart rate. These effects indicate that khat acts through similar central mechanisms as other stimulants [1].

People widely chew khat for different purposes in which, according to the Ethiopian Farmers, chewing enables them to have advantage of staying more time than usual without food intake in food shortage periods due to its anorexic effect. They also use it to get rid of their obesity although there are no empirical data verifying these advantages [12]. Research on weight disorder following khat use in humans is not extensive and several of the available studies have been done only in the context of observational and animal based experiments. Moreover, although comparative studies of amphetamine and khat on physiological and psychological behaviors are extensive, little is known about the consistent and variable effect of khat on anorexia.

Experimental investigation of the effect of khat on rodents shows that it reduces weight gain [13]. Importantly, it reduces maternal weight gain in both rats and women who consumed it during pregnancy by decreasing maternal daily food intake [13, 14, 15]. Additionally, the neonates of khat chewer mothers had a significant decrease in length and head circumference parameters in addition to a decrease in birth weight [16]. Independent studies and morphometric analysis showed that khat intake significantly reduces brain weight both in mice

(prosencephalon) and rats (cerebellar) [17, 18]. It is reported that khat (either extract or whole) causes low mean birth weight possibly by affecting fetal growth during pregnancy as well as it retards growth rate by interfering with food absorption [13, 14]. Investigation on guinea pigs demonstrated that placental insufficiency is observed due to the vasoconstriction effect of norpseudoephedrine which reduce the placental blood flow that impair fetal growth [16]. Similarly, a study made in Ethiopia by Shewamene and Engidawork (2014) on rats showed that subacute khat exposure was associated with reduction in body weight, which can be attributed to either kidney damage, malabsorption, or affecting plasma leptin level [19]. Additionally, some studies reported that CNS tolerance is usual in khat users while other findings claimed that tolerance is not usual probably due to the physical limits on the amount that can be chewed [14]. Thus, the present study investigated the effect of acute and subchronic administration of crude khat extract on anorexia and weight and development of tolerance in mice

2. Materials and Methods

2.1 Plant material

Catha edulis leaves (2000 g) were purchased fresh at a local market in Aweday, 515 km east of Addis Ababa, Ethiopia. The fresh bundles were packed in plastic bag and transported in an icebox to the laboratory. The fresh leaves were then immediately kept at -20 ºC. The plant was identified by a taxonomist and a voucher specimen (001) was deposited in the National Herbarium, College of Natural Sciences, Addis Ababa University.

2.2 Experimental animals

A total of 50 adult albino mice of both sex were used for the whole experiment and they were purchased from the Ethiopian Health and Nutrition Research Institute. They were 6-8 weeks of age and had weights ranging from 20 to 36 g. They were housed in plastic cages with standard wood chip bedding and had access to food and water *ad libitum*. Light was in its natural cycle (for a 12 h on and 12 h off). All experimental procedures on mice were done after 4:00 pm. Care and handling of the mice was performed according to the guidelines given by OECD [20, 21].

2.3 Preparation of crude extract

The freeze-dried plant was finely minced, weighed and placed in Erlenmeyer flasks each wrapped with aluminium foil to avoid light induced decomposition. Chloroform (1350 ml) and diethyl ether (4050 ml) (1: 3 v/v) were added to cover the minced leaves. The resulting mixture was stirred using a rotary shaker at 120 rpm and 20 ºC for 72 h. The mixture was filtered initially using cotton gauze to separate the big particles, followed by Whatman No. 1 filter paper to get rid of fine particles. Initial evaporation was achieved using a rotavapour (120rev/min) under controlled temperature (40^{o}c). Since the mixture could not become completely dry we used a lyophilizer; then it dried. The dry extract was weighed by analytical balance and the yield was 2.6 %. This yield was much more than previously done extractions. The reason could, most probably, be due to the time of purchasing of the fresh leave (it was raining)since the environment and climate condition affects the *general appearance of the khat. The extract was then kept in a tightly sealed material at -20 ºC until used*

2.4 Grouping and dosing of animals

The animals were randomly divided into 4 groups, each comprising eleven animals. Group I served as negative control and was administered with Tween 80 in distilled water (3%, v/v). Group II-IV were administered three dose levels of khat extract: Low-100mg/kg (K100), Moderate-200mg/kg (K200) and High-300mg/kg (K300). The various doses for the khat extract were selected based on previous reports [22, 23] and then administered orally using gavages based on both their body weight and group. The control animals were given the same volume of the vehicle (0.5ml) throughout the month.

Throughout the experimental period, all drug sample solutions were made fresh and sample containers including syringes were covered with aluminium foil to avoid light decomposition. Since the weight of animals was necessary to determine the dose of khat extract, experimental mice were taken from their cage and weighed using electrical digital balance. A less stressful method was employed for oral administration of the extract in mice using gavage. This route was used since chewing is the most common mode of administration for humans [1] and pharmacokinetic studies showed that cathinone and cathine are absorbed from the stomach [11].

The dose of the extract required was determined according to the weight of mice. Total weight of mice taking the same dose of extract or the same group was determined for ease of dose calculation. Once total amount of extract determined was calculated, the extract was reconstituted with 3 % v/v Tween-80 in distilled water. The volume of reconstituted fluid was adjusted so that the maximum volume administration should not exceed 1ml as recommended by OECD guidelines [24, 25, 26]**.** This procedure was repeated everyday at similar time (after 4:00 pm) each day for 30 days of experiment based OECD guidelines and previous studies made at subchronic level [20, 21, 27].

2.5 Weight change measurement

The weight of each mice was taken daily for the first week then every other days, since there was no daily difference in their weight starting from the first week.

For the ease of comparison, weight at day one (labeled as weight 0), at day four (labeled as weight 1), at the end of week 1 (labeled as weight 2), at the end of week 2 (labeled as weight 3), at the end of week 3 (labeled as weight 4) and at the end of week 4 (labeled as weight 5) was taken.

2.6 Statistical analysis

Data obtained from the study are presented as mean ± standard error of mean. Data Analysis was performed using Statistical Package for Social Science (SPSS), version 16.0. Data from weight measurement was analyzed by one way ANOVA followed by Tuckey post hoc test. The significance was set at $p<0.05$.

3. Results

3.1 Qua*litative Observations*

After the first day of crude khat administration, the animals showed obvious signs of intoxications. All mice in the khat group appeared to lose their skin hair. All of the crude khat administered animals were vulnerable to feed their pellet after the first day of the extract administration and started to lose their weight. Subsequently, eight mice died due to anorexic effect of the extract although the other started to regain their weight after they were fed powdered pellet instead.

3.2 Quantitative Observations

The effect of crude khat extract on weight was measured using One-way ANOVA. As shown in Fig 1, the weight of CON mice increased throughout the period of administration, while the weight of khat-treated animals decreased up to day four (weight 1) then started to regain their weight and increases up to the weight of weight 5 (day 30). There was significant weight difference among groups at day 4 (Delta1: weight at day 4 minus weight at day1) (F (3, 32) =94.539) (Fig 2). Post hoc test with Tukey procedure revealed that the weight of CON group has significantly increased when compared with K100, K200 and K300.

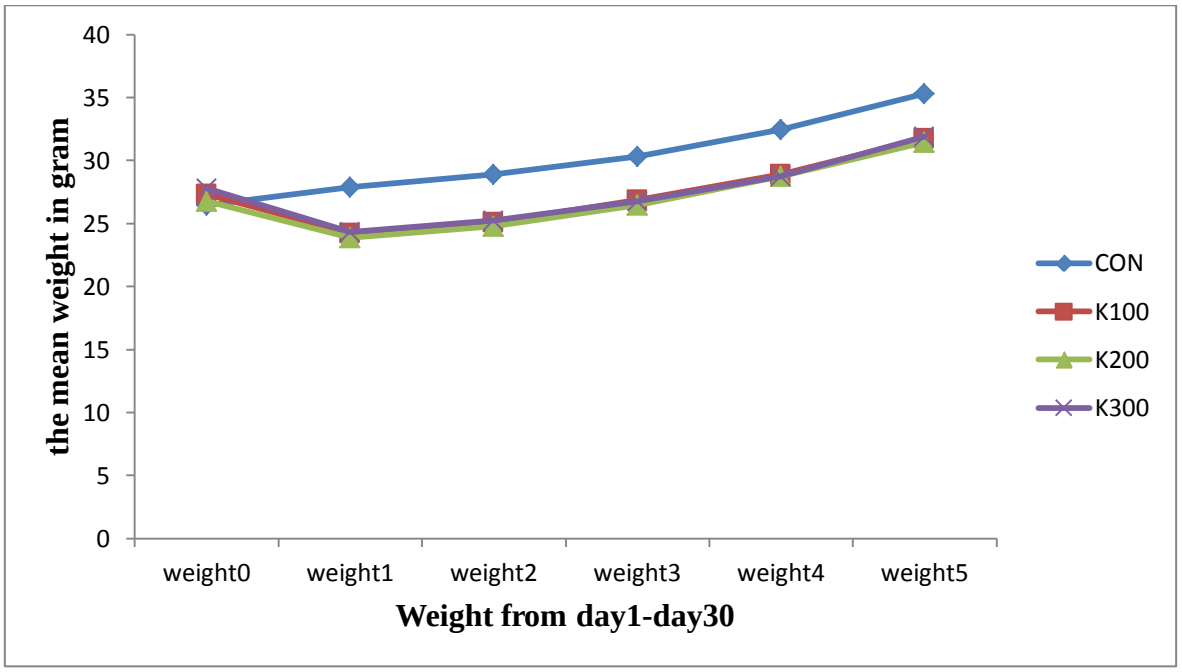

Figure 1: Mean weight from day 1 to day 30 in gram for all groups. Weight 0: weight at day 1, Weight 1: Weight at day, Weight 2: weight at the end of week1 (day 7), Weight 5: weight at day 30. Values are mean ±SEM (n=9); CON: Control group, K100:100mg/kg khat extract, K200: 200mg/kg khat extract, and K300:300mg/kg khat extract.

One –way repeated measure of ANOVA for the Delta 2(weight difference between weight 5 and weight1) shows there is no significant weight difference among groups (F (3, 32) =0.000) (Fig 2). Post hoc test with Tukey procedure revealed that the weight of CON was not significantly different from the other groups (K100, K200 and K300).

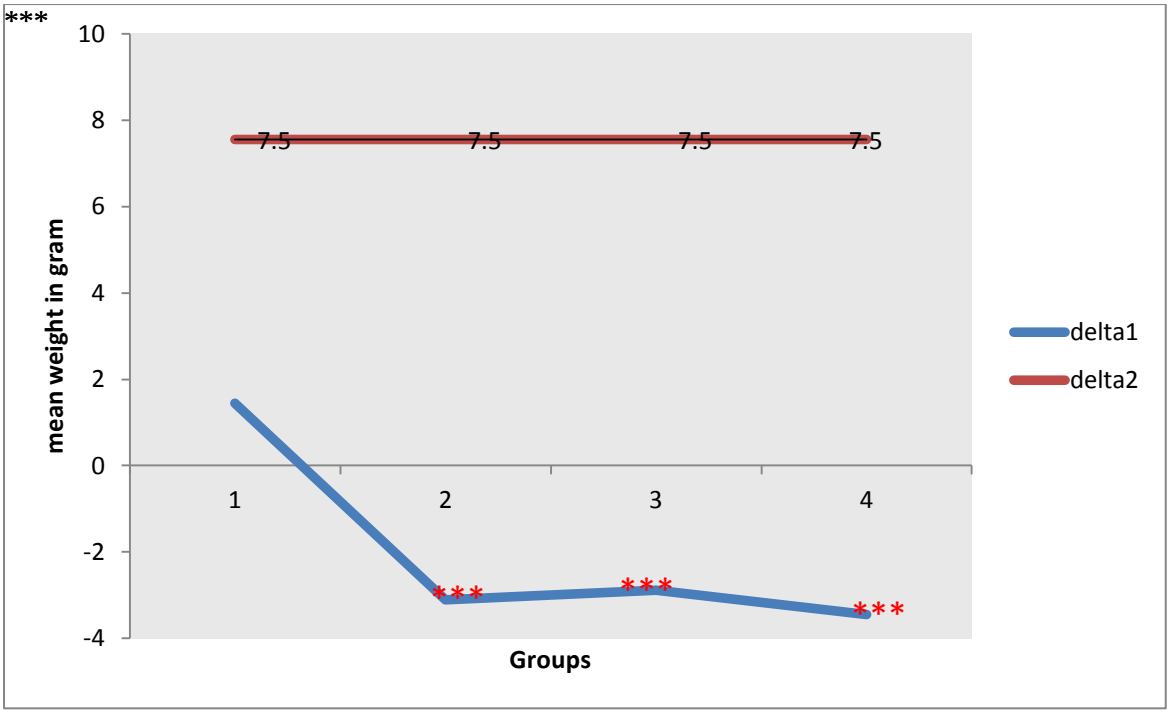

Figure 1: Mean weight differences. Values are mean ±SEM (n=9); all values are mean ± SEM (n=9) and statistical analysis was performed using one-way ANOVA; ***: $p<0.001$, CON: Control group, K100:100mg/kg khat extract, K200: 200mg/kg khat extract, and K300:300mg/kg khat extract. Delta 1 is the difference between weight at day 4 (weight 1) and weight before administration of the extract and the vehicle at day 1 (weight 0). Delta 2 is the difference between weight at day 30 (weight 5) and weight at day 4 (weight 1).

4. Discussion

In the present study the effect of acute and subchronic administration of crude khat extract on anorexia and weight was evaluated using mice. Most of the studies that evaluate the effect of khat extract on weight and /or anorexia were performed using rats, but produced conflicting results. The major finding of the present study is that the effect of acute and subchronic exposure to khat does have a variable effect on weight. During the four consecutive days of acute exposures, the weight of CON group has significantly increased when compared with khat-treated groups. On the other hand, the weight of khat-treated groups decreased in those four days which appears to be followed by recovery and increase of weight during subchronic administration. The weight difference of CON was not significantly different from the other groups at the end of subchronic administration. Thus, it is plausible to say that acute exposure causes significant weight decrease while subchronic exposure would cause development of tolerance significantly.

Studies performed with substances that have resemblance to cathinone, such as amphetamine, have shown to have initial anorexic effect which is later followed by tolerance [10]. It has been reported that the effects of a portion of khat are very similar to those of about 5-mg amphetamine. Cathinone , for instance, releases catecholamaines from pre-synaptic storage sites resulting in CNS stimulation [14]. There is clear-cut evidence that both cathinone and amphetamine stimulate the CNS and suppress appetite [6]. Even if the present result

showed that khat is much likely to cause tolerance since there was an increase in weight in the last two weeks, there are controversies regarding the level of tolerance caused by khat. This has made the nature of khat dependence to remain under active debate and accumulating evidence also indicates the existence of a withdrawal syndrome and a low level of tolerance [1]. Subsequently, other studies show that khat, in comparison with amphetamine, is much less likely to cause tolerance. In particular, the stimulant CNS effects of khat do not seem subject to the development of tolerance, but some degree of tolerance to insomnia and anorexia has been observed in most chronic khat chewers [4]. But others reported the development of tolerance to the effects of cathinone is more rapid than to that of amphetamine, and there is cross-tolerance between cathinone and amphetamine [28].

The effect of khat on food intake then on weight can be mediated by its release of biogenic amines such as noradrenaline, dopamine and serotonin since it is well documented that dopamine and serotonin cause loss of appetite and induce anorexic effect. Drugs like ondansetron (5-HT3 antagonists) and chlorpromazine (dopamine antagonist) which antagonize the effects of dopamine and serotonin have been approved for the prevention and treatment of nausea and vomiting [32, 33]. It is also found that administration of noradrenaline or treatment with ß-agonists in mice decreases plasma leptin levels and transcription in white adipocytes which results in reduction of body weight [29].

Both cathinone and amphetamine increase the activity of the noradrenergic, dopaminergic and serotonergic transmisson by releasing them from presynaptic storage sites and subsequently inhibit their uptake, thereby increasing temporal and spatial presence of these neurotransmitters at the presynaptic receptors [1, 3, 28, 30]. The sympathomimetic properties of khat are due to peripheral noradrenalin-releasing properties of khat amines, which potentiate noradrenogenic transmission. It was also found to inhibit neural uptake of noradrenalin [9]**.** At peripheral sites cathinone, norpseudoephedrine, and norephedrine are about equipotent with regard to induction of release at noradrenergic nerve terminals [31]. Cathinone is not considered a direct dopamine agonist but rather a presynaptic releaser and re-uptake inhibitor of dopamine [10]**.** Although cathinone is the most potent with regard to induction of release at CNS dopamine terminals, the other two main khat alkaloids are also induce dopamine release [31]. Importantly, chronic administration of either the whole extract (since both cathine and norephedrine also have effect) or cathinone (100 mg/kg) results in a significant depletion of dopamine in several brain areas, particularly on the nigrostriatal dopamine terminal projections [10, 31]. So far there is no clear cut evidence on the role of serotonergic and/or other pathways in the stimulatory effect of cathinone [10]. It has been suggested that cathinone, like amphetamine, releases serotonin in the CNS [8, 10, 28] although some investigators have reported that levels of serotonin in rat brain are not altered by repeated administration of cathinone [3]. So it can be suggested that the anorexic effect of crude khat extract is due to its induction effect on all noradrenalin, serotonin and dopamine or on either one of them. Subsequently, the development of tolerance after its anorexic effect is plausibly due to depletion of dopamine after repeated administration of the extract.

5. Conclusion

This study showed that acute and subchronic exposure of mice to khat had significant effect on initial loss of

body weight during acute exposure even if tolerance developed finally to the anorexic effect of this extract.

6. Limitations of the study

We were trying to compare the concentration of cathinone in the extract with standard cathinone but it became impossible to import standard cathinone from other countries. Most people in Ethiopia chew khat together with alcohol, caffeine or nicotine. So, it is expected to identify the combinational effects.

7. Recommendation

- The effect of cathinone that is fractionated from the crude extract of khat on weight needs to be investigated.
- Neurotransmitters level (dopamine, serotonin and noradrenalin) needs to be determined.
- The effect of crude khat extract on genetic and epigenetic level needs to be investigated
- The effect of nicotine and crude khat extract together on weight needs to be investigated as a new direction.
- The effect of alcohol and crude khat extract together on weight needs to be investigated as a new direction.
- The effect of caffeine and crude khat extract together on weight needs to be investigated as a new direction.

Conflict of Interest

We declare that there are no conflicts of interest to disclose.

Acknowledgement

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