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Gross Morphometric Indices of the Cerebral Cortex of the Wistar Rat Following Chronic Use of Khat

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Abstract: ***Our objective was to evaluate the dose-dependent effect of khat on body and cerebral cortical indices among wistar rats on chronic administration of khat.Young adult wistar rats were randomized into controls, and three experimental groups to receive 500mg/kg, 1000mg/kg and 2000mg/kg crude khat extracts respectively. After 6 weeks of khat administration, their body and brain weights were taken, as well as cortical thickness, length and supero-inferior dimensions. The mean body weight for control group was 414.36g, and was significantly higher than rats fed on 1000mg/kg and 2000mg/kg crude khat extracts. A similar trend was observed in absolute brain weight, maximum cortical length, cortical width and supero-inferior diameter of the brains. This study found that khat has significant effect in lowering body and brain weight as well as cortical indices. This may be a marker of the effects of khat at the cellular level.***

Keywords: Khat, brain indices, chronic, wistar rats.

1. Background to the Study

The cerebral cortex is important in control of movement, coordination, cognition among other important functions. It is agreed that morphometric study of gross organs may shed light on functional correlation, and give information on subtle molecular changes that occur in body organs [1].

Khat exerts its main effects through two amphetamine-like alkaloids, cathine and cathinone. In some communities, khat is believed to possess many health benefits, among them anti-diabetic and anti-obesity effects associated with suppression of appetite [2,3]. Further, khat has been found to have cardiovascular effects (elevated blood pressure, tachycardia), gastrointestinal effects (anorexia, constipation) and potent central nervous system effects (insomnia, dependence, neurocognitive perturbation) [4].

The cardiovascular, gastrointestinal and nervous effects are all interlinked functionally. Chronic administration of khat causes dopamine depletion, accompanied by a reduction in number of dopamine transporters [5, 6].

In the rat model, Khat use reduces body weight [7, 8]. If administered in high doses over long periods, a change in body weight is observed [9]. A drop in leptin levels, which has receptors in hypothalamus, has been linked with the weight changes [10]. Khat suppresses appetite by increasing synaptic concentration of norepinephrine and dopamine in hypothalamus, thus disrupting the central nervous regulation of food intake.

Structural damage induced by khat has been reported in certain brain areas such as the cortex [7] and cerebellum [8]. This is attributable to changes in body weight, poor nutritional intake affecting neuronal growth. This has effects on complex neurocognitive roles played by the brain.

However, there remains paucity of data on dose-related reduction of body and brain weight on chronic administration of khat. This could be an easier marker of brain effects of khat use.

2. Materials and Methods

Young adult male Wistar rats, aged 2-3 months, weighing 200-300 grams were used in this study. They were randomized into four groups of 10 each (control, K500, K1000 and K2000) to correspond with those used as controls, those that received 500mg/kg, 1000mg/kg and 2000mg/kg body weight khat extracts respectively.

Khat preparation: We purchased fresh khat leaves from Maua market in Meru and prepared crude extract using a validated method. After weighing, each bunch of khat was chopped to homogenize the sample and blended with 125ml of sterile distilled water. The blended mixture was then transferred to 40ml falcon tubes and centrifuged at 7000rpm for 6min.The supernatant was then transferred into 100ml bottles covered with aluminium foil to minimize exposure to light and stored at refrigerated conditions of 2°C awaiting lyophilisation. Supernatant from Khat extract was then dispensed in volumes of 3ml into vials for lyophilisation. The vials were first frozen at -80°C for 2 hrs then freeze dried under vacuum at 0.103mBar for 24hrs.

The control rats were fed on normal diet, while experimental groups were fed on normal diet and khat extracts using oral gavage for 6 weeks.

The animals were sacrificed using intraperitoneal ketamine and xylazine and their brains promptly removed. With the aid of a scalpel blade, the head was decapitated at the occipito-atlantal junction, skinned and with the aid of scissors and saw, the skull bones chipped off to expose the brain in the cranium with the olfactory bulb extending towards the nasal bone areas. The meninges were carefully dissected away from the frontal region, with the tentorium cerebelli nipped off to free the dorsal hindbrain region.

The falx cerebri was then removed in between the bulbs to prevent breakage of the olfactory bulb from the rest of the brain structure. Ventrally, the meninges and the cranial nerves were severed to free the entire brain from the cranium. Gross examination was carried out visually with the aid of a dissection microscope, scalpel and forceps.

The following gross parameters were measured

1) Body weight (g): Weight of the rat in grams
2) Absolute brain weight (g): Weight of the whole dissected brain without the meninges
3) Relative brain weight: Ratio of brain weight to the body weight
4) Maximum cerebral cortical width (mm): maximum length across the most lateral portion of the parietal lobes of the cerebral cortex
5) Maximum cortex length (mm): maximum length from the tip of the frontal lobe to the caudal most portion of the occipital lobe of the cerebral cortex

All the above parameters were measured to the nearest 0.01 units (grams or mm). Figure 1 below illustrates how some of the dimensions were measured

Figure 1: Method of measurement of the antero-posterior diameter of the rat brain to the nearest 0.01mm

3. Results

The adult brains displayed putative gyrencephaly, with prominent olfactory bulbs, an oblong cerebral cortex and prominent cerebellum and medulla as shown in Fig 2 below.

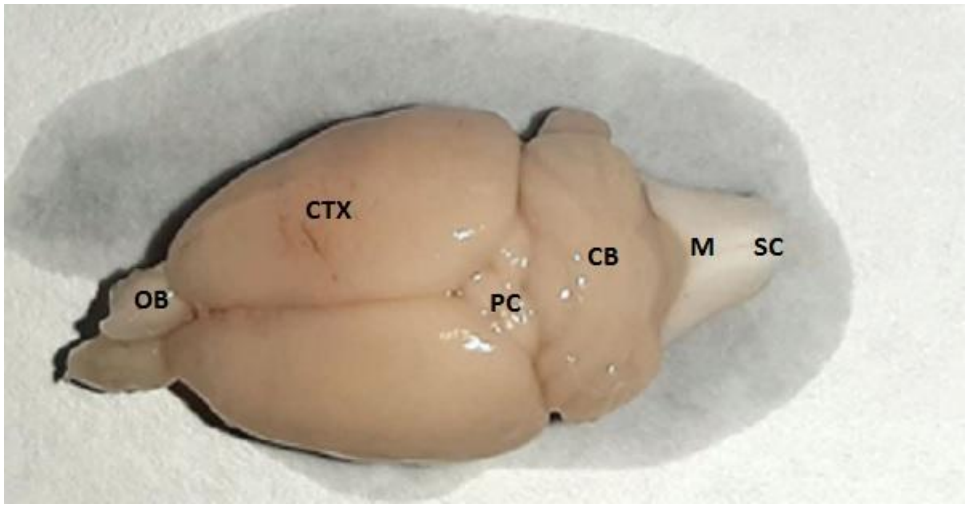

Figure 2: Extracted rat brain showing a gyrencephalic brain and major structures like cerebral cortex (CTX), olfactory bulb (OB), posterior colliculi (PC), medulla (M), spinal cord (SC) and cerebellum (CB)

The mean body weight of the control rats was 414.36g, and there was a decrease in body weight with increasing doses of khat. The mean body weights in group 2 (fed 1000mg/kg khat) and group 3 (2000mg/kg khat) were significantly lower than controls ($p< 0.002$ and $p< 0.0018$) respectively. Table 1 below and Figure 3 illustrates the body weights of the controls and experimental groups.

Control rats had an average brain weight of 2.04g, and showed a decrease across the experimental groups, with group 3 (2000mg/kg) having a significantly lower brain weight (mean 1.88g) ($p<0.024$). However, the relative brain weight was lowest in controls (0.5%) and highest in group 2 rats (0.69g), and the difference was statistically significant ($p<0.04$).

The maximum cortical length was largest in controls (16.72mm) and smallest in group 3 rats fed on 2000mg/kg khat (15.83mm) and the difference was statistically significant ($p <0.03$). The cerebral cortical width, and supero-inferior thickness of brains of controls were significantly higher than group 2 and 3. In all brain indices except the relative brain weight, there was a gradual decrease across the groups.

Table 1: Mean body weight and fixed brain weights of controls and experimental rat groups after 6 weeks of khat feeding

Parameters	Body weight (g) ±SEM	Brain weight (g) ±SEM	Relative brain weight (%) ± SEM
Controls	414.36±16.32	2.04±0.05	0.5
Group 1 (500mg/kg)	314.64±10.98	1.97±0.06	0.63
Group 2 (1000mg/kg)	305.55± 21.18[a]	1.92±0.03	0.69[c]
Group 3 (2000mg/kg)	290.64±19.39[a]	1.88±0.03[b]	0.68

[a]The average body weight of group 2 (1000mg/kg) and group 3 (2000mg/kg) were significantly lower than controls,[b] the absolute brain weight of group 3 rats (2000mg/kg khat-fed) was significantly lower than control rats, and [c]the relative (%) brain weight of group 2 (1000mg/kg khat-fed) was significantly higher than control rats.

Table 2: Mean cerebral cortical parameters of controls and experimental rat groups after 6 weeks of khat feeding

Parameters Rat groups	Maximum cortical length (mm)	Maximum cortical width (mm)	Supero-inferior brain thickness (mm)
Controls	16.72	15.77	10.02
Group 1 (500mg/kg khat)	16.43	15.42	9.85
Group 2 (1000mg/kg khat)	16.03	15.38	9.66
Group 3 (2000mg/kg)	15.82	15.23	9.53

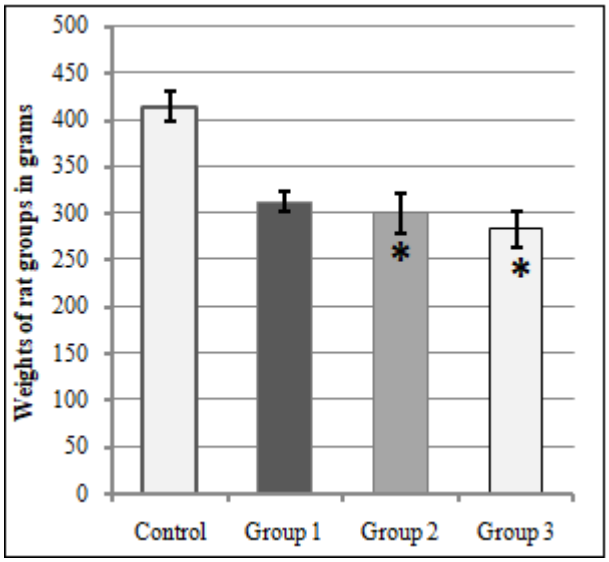

Figure 3: Average weights of controls and experimental rat groups after 6 weeks

*The weights in group 2 and 3 are significantly lower compared to control group at 6 weeks ($p < 0.002$, $p < 0.0018$ respectively).

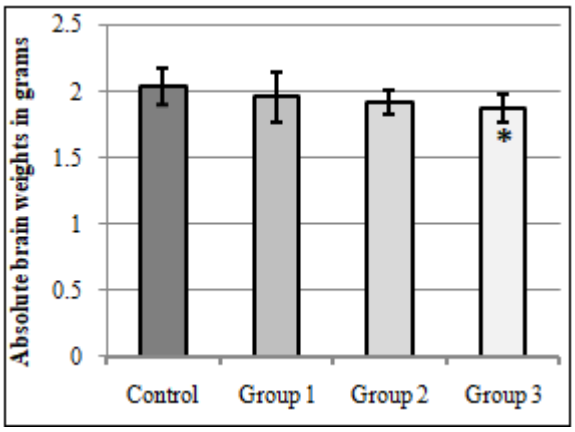

Figure 4: Absolute brain weights of controls and experimental groups at 6 weeks

*Absolute brain weight of group 3 rats significantly lower compared to controls ($p<0.0024$)

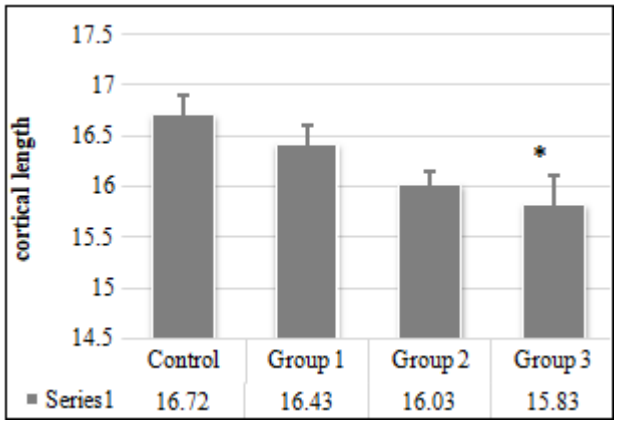

Figure 5: Bar chart showing maximum cortical length of controls and experimental groups

*Maximum cortical length of the rats fed on 2000mg/kg Khat was significantly lower compared to controls ($p<0.03$)

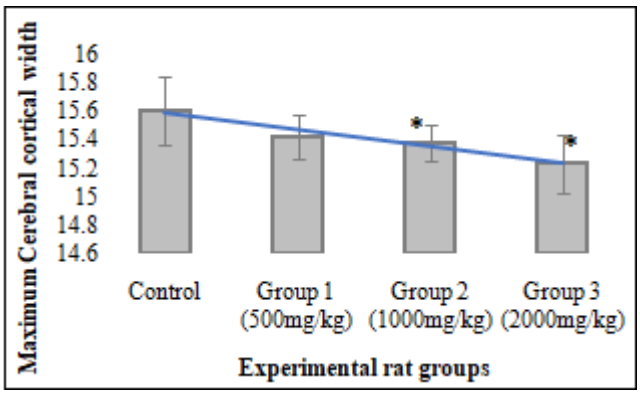

Figure 6: Cerebral cortical width of controls and experimental rat groups

*Cerebral cortex width of group 2 and group 3 rat groups was significantly less than rats that were fed on normal diet ($p<0.015$ and 0.012 respectively).

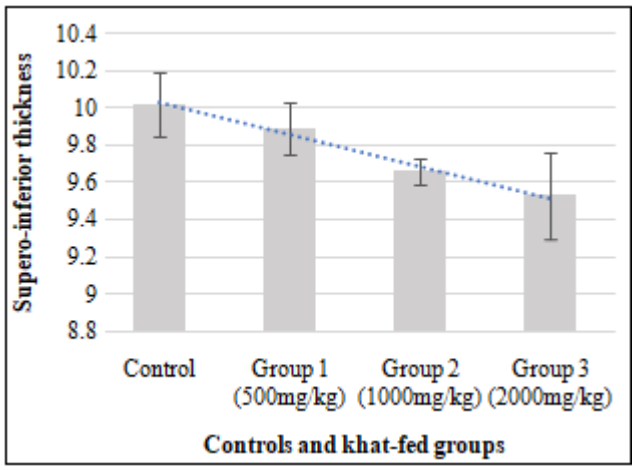

Figure 7: Supero-inferior brain thickness of controls and rats fed on khat

*The supero-inferior thickness of control rats was significantly larger compared to rats fed on 1000mg/kg khat ($p<0.0026$) and those fed on 2000mg/kg khat ($p<0.0018$)

4. Discussion

Morphometric data is invaluable in providing a glimpse of structure-function relationship in body organs as a result of exposure to drugs and substances [11]. Further, dose-dependent changes in brain parameters add valuable information on correlation with micro-anatomic as well as imaging studies.

We found a mean brain weight of 2.04g in control group, which is in keeping with what has been reported in a previous study [8]. The brain weights of corresponding experimental groups decreased, and the rats fed on highest dose of khat had the lowest brain weight. Abebe et al[8] reported a statistically significant decrease in brain weight of 10.04% between khat-fed rats and their corresponding controls. Similar changes in brain weight have been reported in past studies [12].

The decrease in brain weight may be attributable to amphetamine-like effect of cathinone on the release of norepinephrine in the brain, which activates the satiety centres [13]. The result is delayed gastric emptying, suppressed appetite, and hampered neuronal nutrition.

We found a decrease in gross cortical parameters in experimental rat groups compared to controls, with the decrease most significant in higher doses of khat. This decrease observed in the current study could be partially explained by neuronal effects as has been observed before [14, 15]. Neuronal loss in the prefrontal cortex and cerebellum and other brain areas are well reported.

Neuronal death through glutamate-induced excitotoxicity in the cerebral cortex, with release of intracellular calcium and membrane damage is a potential mechanism in khat-induced changes.

5. Conclusion

In conclusion, khat has been found to cause lower body weights and a decrease in brain cortical indices in experimental rats, and these findings shed more light on the potential neurocognitive perturbations associated with khat and the likely corresponding doses.

Conflicts of interest: None

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