Assessment of the Antioxidants and Genotoxicity of *Catha edulis* (khat) Crude Extract Sub-Chronic Administration in rats

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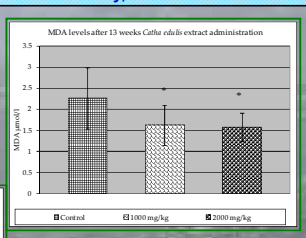
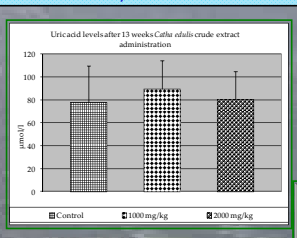
Introduction

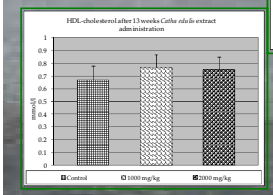
The leaves of *Catha edulis* (Celastraceae) (khat), a plant growing wild and cultivated in Eastern Africa and Southern Arabia is chewed for its stimulating and sympathomimetic effects. The alkaloid fraction of khat is very efficiently extracted by chewing, and the major compounds are absorbed in the oral cavity (Toennes *et al*., 2003). Increase in micronucleated buccal cells were observed in khat chewers and a centromeric signal of aneuploidogenic effect among heavy khat chewers (Kassie *et al*., 2001). Leaves extract administration was found to inhibit RNA and DNA synthesis in the neurons of chick embryo (Hammouda, 1971) and reduce DNA and RNA contents in liver and brain tissue homogenates in rat (Hondet *et al*., 1984). Meanwhile, cytotoxicity and mutagenicity to mammalian cells was reported after methanolic khat leaves extract treatment (Al-Ahdal *et al*., 1988). Recently the effect of whole khat extract on three leukemia cell lines (HL-60, Jurkat, and NB4 cells) was reported to be cytotoxic and induced a rapid cell death effect (Dimba *et al*., 2003) and induced apoptosis through a mechanism involving activation of caspase-1, -3 and -8 (Dimba *et al*., 2004). The aim of this work was to highlight the genotoxic effects of *Catha edulis* subchronic administration in rats.

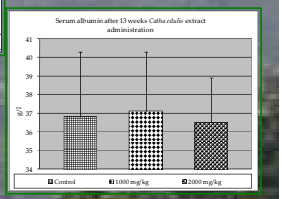
Methodology

The study design is an adaptation of OECD Guideline No. 408 for a Repeated Dose 90-day Oral Toxicity Study in Rodents. In this study two treated groups (n=13 rats in each) and control group (n=10 rats) were used, 1000 mg/kg body weight and 2000 mg/kg body weight, fed crude khat extract for 13 weeks. Two genotoxicity assays were used in this study namely, Comet Assay and Chromosomal Aberrations Assay, in addition to biochemical analysis of MDA as measured in the form of TBARS and Uric acid, Albumin and HDL as antioxidant substances.









Chromosomal Aberrations Assay

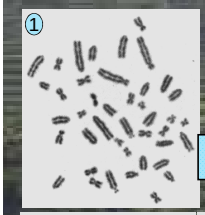
Comet Assay (SCGE)

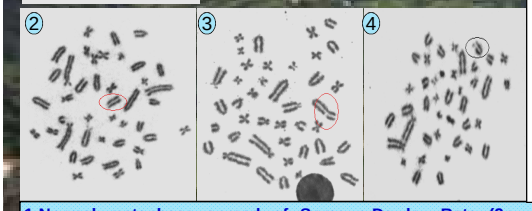
Peripheral blood lymphocytes were cultured in RPMI 1640 with PHA for 72 hr, Colcemid added before 1 hr. Cells harvested, fixed with EOH : Acetic acid, spread on microscope slide, stained with Conventional Geimsa's Stain and Chromosomal Aberrations scored after counting well spread 50 metaphases.

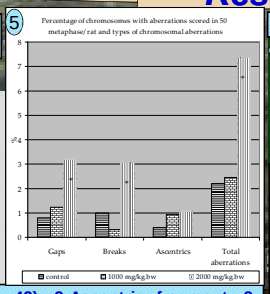
-Slide preparation (LMA), alkaline Unwinding of DNA in P. Buffer pH>13, 20 min.
-Electrophoresis(33 V, 30 min, 300 mA), SYBR Green Staining and Image capture: Fluorescent Microscopic Detection for DNA damage

Results

Microscopic examination of Geimsa's stained air-dried chromosomes spread







1-Normal metaphase spread of Sprague-Dawley Rats (2n= 42), 2-Ascentric fragment, 3-Chromatid break and 4- Chromatid gap. 5-Distribution of chromosomal aberrations in different groups (*) P<0.05.

Comet images scored visually from 0 (No DNA damage) to 4 (DNA Completely in the head)

Mean level of oxidative DNA damage after 13 weeks of *Catha edulis* crude extract administration in rats (+ve control of H_2O_2 treated cells included with each run).

	Control	1000 mg/kg	2000 mg/kg
DNA damage (%)	5.20±0.89	6.63±0.88	5.88±0.55

Scoring done by counting 50 nuclei in duplicate. The results showed No significant differences were observed in the treatment groups compared to the control group.

Discussion

In this study, we attempted to probe the long-term, sub-chronic (13 weeks) effects of khat consumption in rats by mimicking the regular khat-chewing activity of human. Feeding rats for 13 weeks showed the presence of clastogenic effect of dried khat leaves extract in a high dose (2000 mg/kg body weight) that could be attributed to some of the leaves content. These results showed the presence of chromosome aberrations in the form of chromatid gaps and breaks and acentric fragments. To detect DNA double strand breaks, we used the alkaline version of the Comet Assay. Our study failed to show an effect of khat (*Catha edulis*) leaves crude extract administration on DNA migration of rat lymphocytes in the comet assay. These results were found to be in controversy with the chromosome aberration assay results, in which the main aberrations reported were chromatid gaps and breaks. This discrepancy could be due to the lower sensitivity of comet assay than chromosome aberration assay (if we consider gaps as of biological significance) and the type of chromosome aberrations observed. Meanwhile there is no published work correlating the comet assay to the proportion of aberrant chromosomes. In addition a reduction in the lipid peroxidation product, MDA (detected as TBARS) was observed in the two groups compared to the control group. Further studies needed to highlight the clastogenic effects of *Catha edulis* using chromosomal aberrations assay and micronucleus test in vivo using bone marrow as well as in vitro using human peripheral blood lymphocytes. In addition these results revealed the antioxidant properties of *Catha edulis* after oral administration in rats for 13 weeks, that could be attributed to the high contents of polyphenolic compounds of *Catha edulis* leaves.

References

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