Genotoxic Effects of Catha edulis (Khat) Extract on Mice Bone Marrow Cells

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Abstract

Khat (Catha edulis) is a widespread habit that has a deep-rooted sociocultural tradition in the Horn of Africa and southwestern of Arabian Peninsula causing many social and economic problems. The genotoxic effects of methanolic extract of Khat (Catha edulis) leaves were investigated. Our results demonstrated a significant increase in sister chromatid exchanges SCEs in all treatments. This increase was more at higher concentrations than those occurred in lower concentrations. Moreover, Khat induced various types of chromosomal aberrations in mice bone marrow cells. These aberrations include: broken, sticky and ring chromosomes and disturbed metaphase and anaphase. The percentages of these aberrations increased with the increase of both concentration and the exposure time. Other types of aberrations were also noticed but in very low frequencies.

1. Introduction

Catha edulis (Khat) leaves are used by millions of people worldwide, mainly in Africa and the south west of Arabian peninsula causing many social and economic problems (Osborne, 1983). 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). Its stimulating effects as well as the psychological reaction induced among users have been reported by Kalix (1990). It was believed that the problem might be social rather than medical. But in some regions, a large proportion of population was spending great deal of the family income on Khat rather than on food, with the consequence that the consumers and their families suffered from malnutrition and weakness (Kabarity and Mallalah, 1980). Khat chewing during pregnancy may be one of the factors contributing to infant mortality in communities, where Khat is commonly chewed as well as Khat consumption affects the potency of male sexuality by affecting spermatogenesis and plasma testosterone concentration (Mwenda et al., 2003).

Toxicological evaluation of Catha edulis leaves has been reported by Al-Habori et al., (2002). Moreover, the toxicological potential of Khat has been reported by Carvalho (2003). The detrimental effects of the active principle Khat on man and animals have been described by Kalix and Khan (1984), and its mutagenic activity has been demonstrated by Hannan et al. (1985). Moreover, fresh leaves are chewed to produce an amphetamine like alkaloid, known as cathinone (AL-Ahdal et al., 1988). The active principle from Catha edulis (Khat) induced clastogenic effects in bone marrow of mice (Tariq et al., 1987).

Anti-gastric ulcer and anti-inflammatory activities of Khat have been also reported by Al-Meshal et al., (1983, 1985). The pharmacological activity of Khat has been described by several workers (Balint et al.1991; Kalix, 1991; Nencini and Ahmed, 1989 and Widler et al., 1994). The biochemical activity of Khat has been described by Ahmed and El-Qirbi (1993) and its psychological effect

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has been reported by Mclaren (1987), Dhadphale and Omolo (1988).

Antimicrobial and cytotoxic activity of Khat extracts have been reported by Elhag et al. (1999). Moreover, it has been shown that cathinone isolated from Catha edulis (Khat) induced mitodepressive effect on the meristematic region of Allium cepa root tips (Al-Meshal, 1987).

Because of its adverse effects on human, Khat was classified by the World Health Organization in 1962 as a 'substance of abuse' (Kabarity and Mallalah, 1980; Giannini and Castellani, 1982). Indeed, a large number of medical reports have been reported in Khat chewers (Nencini et al., 1986; Granek et al., 1988; Eriksson et al., 1991; Widler et al., 1994) such as oral cancer (Soufi et al., 1991). Moreover, the incidence of head and neck squamous cell carcinoma seems to be relatively high, especially the oral squamous cell carcinoma, which may be considered as an important contributing factor (Nasr and Khatr, 2000). Accordingly, investigation of harmful effects i.e. genotoxic effects of Khat should be done by sensitive and reliable method for detecting its genotoxicity.

It has also been reported that Khat induces cytotoxic effects in cells (Al-Ahdal et al. 1988; Al-Meshal et al. 1991; Al-Mamary et al. 2002; Dimba et al. 2003) in lymphoid tissue and in the liver and kidney of rabbits (Al-Meshal et al., 1991; Al-Mamary et al., 2002). Recently, the effect of 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). It also induced apoptosis through a mechanism involving activation of caspase-1, caspase-3 and caspase-8 (Dimba et al., 2003). The dried powder of Catha edulis (Khat) leaves was extracted continuously for 24h in a soxhlet extraction apparatus. The air dried powder of Catha edulis was extracted for different periods of time after a single intraperitoneal injection. All dosing solutions we administered were at a volume of 0.1 ml/kg body weight (b.w.). Animals in the negative control groups received an equivalent volume of normal saline. Mitomycin c (MMC) was used as positive controls. Metaphase bone marrow cells were prepared for mitotic investigation by the classical method. The preparations were stained with Giemsa solution, pH 6.8, as described by Allen et al. (1978). Slides were also scored for chromosome aberration. Evaluation of genotoxic effects of Catha edulis (Khat) extracts, in terms of their capacity to induce various types of chromosome aberrations, was studied.  

2. Material and Methods

The dried material of Catha edulis (Khat) leaves was ground and powdered using an electric grinder. The powdered material was then extracted using a soxhlet extraction apparatus. The air dried powder of Catha edulis was extracted continuously for 24h in a soxhlet extraction apparatus with a range of solvents, with n-hexane (to separate lipids and terpenoids); with ethyl acetate (for separation of more polar compounds), and then using methanol (for separation of the polar compounds) as in the extraction procedures according to Ayoub et al. (1989).

A series of concentrations of the methanol extract were prepared 10,25,50, and 100 mg/kg, and their genotoxic effects on bone marrow cells of Swiss male white mice (Mus musculus, 2n= 40) were tested for different periods of time after a single intraperitoneal injection.
3. Results

*Catha edulis* (Khat) extract induced a significant increase in the frequency of SCEs (P<0.001) in bone marrow cells as compared with untreated controls at all three doses (10, 20 and 40 mg/kg) (Table 1). However, no significant difference could be found in the frequency of SCEs among treated groups. Moreover, the potency of *Catha edulis* (Khat) on induction of SCEs was significantly lower than that caused by the positive control (MMC) by almost the 50% in all treatments (Table 1).

Statistical analysis of the data (t-test, P< 0.001) revealed that there is a significant difference between Khat treated groups at different concentrations and exposure times and the control group (Table 2). Moreover, in the three exposure times, the percentages of chromosomal aberrations in the treated groups were significantly higher than those of the control. These percentages increased with increased concentrations as well as with increased exposure times (see Table 2).

4. Discussion

*Catha edulis* (Khat) leaves are used by millions of people as a social habit, and there is little information about its biological activity (Carvalho, 2003). The aim of this study was to investigate the genotoxic effects of Khat extract as measured by chromosomal aberrations of the following parameters: sister chromatid exchange (SCE) and chromosomal aberration.
Table 1. Effects of Khat extract on the frequency of SCEs in the bone marrow cells of mice.

| Dose (mg/kg) b.w | Cells examined | SCE/ CELL Mean ±S.D.
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Significantly different from control (p < 0.001)

Sister chromatid exchange (SCE) test is widely used in genetic toxicology, and therefore the induced SCE is of great importance (Bruckmann et al. 1999). Moreover, it is considered a sensitive indicator of genetic toxicity (Khalil, 1996).

The present study of Khat extract effect on bone marrow cells of mice has revealed that Khat extract causes significantly increased frequencies of SCEs (P < 0.001) in cells treated as compared with the controls at all three doses (10, 20, and 40 mg/kg) (Table 1). However, no significant difference could be found among treated groups. Similar increase in the frequency of SCEs induced by alkaloids has been reported by Das et al. (2004) on their study using Sanguinarine (SG), a benzophenanthridine alkaloid. They found that (SG) increased sister chromatid exchange frequencies. It is also reported that the major alkaloid of betel nut, arecoline (ARC) induced a high frequency of SCEs after oral administration (OA) and intraperitoneal injection (IP) in mouse bone marrow cells (Chatterjee and Deb, 1999). Moreover, Boldine which is an alkaloid present in *Peumus boldus* (popularly called “boldo- do-chile” in Brazil) which has healing properties; and is used for the treatment of gastrointestinal disorders induced SCEs when tested in vitro on human peripheral blood lymphocytes (Tavares and Takahashi, 1994).

Khat consumption caused genotoxic effect in humans (Kassie et al. 2001). It also caused cytotoxic effects in bone marrow cells of mice treated with 125, 250, and 500 mg/kg (Qureshi et al. 1988).

SCEs arise from reciprocal exchange of DNA at apparently identical loci of the sister chromatids of a duplicated chromosome in response to a damaged DNA template. The frequency of SCEs in eukaryotic cells is increased by exposure to genotoxic agents, which induce DNA damage that is capable of interfering with DNA replication (Tucker et al. 1993). The significant increase in the frequency of SCEs induced by Khat extract may further indicate the potential interaction with cellular DNA. So, the present data clearly indicates that (Khat) possesses the potential, at least to a limited extent, to cause alterations in cellular DNA in mice cells in vivo.

In the current study, the percentage of chromosomal aberrations in cells after 4 h exposure with the control (DMSO) was 0.24 (Table 2). This percentage was elevated to 0.45 and 0.53 when exposure time was increased to 24 h and 48 h respectively. These results are in accordance with those reported by Tavares and Takahashi (1994) on studying genotoxic potential of the alkaloid boldine in mammalian cell systems in vitro and in vivo using blood samples from healthy people.

Significant differences in the percentage of chromosomal aberrations relative to the control were also observed when alkaloids were applied in almost all treatments. After 6 h exposure, the percentage was 0.74 at the lowest concentration (10 mg/kg b.w.), this was elevated to 3.10 at the highest concentration (100 mg/kg b.w.). This trend of elevation was also observed at the other two exposure times 24 h and 48 h (Table 2).

Referring to the same table, it is clear that the percentage of chromosomal aberrations increases with increased concentration as well as increased exposure time. Similar results were obtained by AL-Zubairi et al. (2008) on studying the genotoxic effect of Khat in rats. However, we observed concentration dependent chromosome aberration frequencies. These results are also in agreement with those reported by Ribeiro et al. (1993) on studying the effect of extracts obtained from *Crotalaria retusa* on mouse bone marrow cells.

Therefore, we can suggest that *Catha edulis* (Khat) contains some mutagenic and potential carcinogenic agents. Culvenor et al. (1962) presented evidence that the effects of alkaloids on cell nuclei are due primarily to their ability to act in the cell as alkylating agents.

Linearly along with increasing concentrations of alkaloids as well as *Catha edulis* (Khat) extract induced the formation of various types of chromosomal aberrations. The most common abnormality is the broken chromosome as shown in Figure1. Chromosomal breaks result from the action on the DNA synthesis (Evans, 1969).

Broken chromosome percentages are increased linearly along with concentrations used; and are also increased through different periods of time in all cases.
This suggests that alkaloidal fraction may contain alkylating compounds (S-dependent agents) that produce aberrations via misreplication (DNA damage happened when a DNA molecule with lesions undergoes DNA replication) (Palitti, 1998). This suggestion is supported by Peter et al. (2002), who found a variety of alkaloids in Astraceae family plants that produced genotoxicity.

Sticky chromosomes occurred at a high percentage with extract treatments (Figure 2). The percentage of this type of aberration is also increased linearly with the concentration and exposure time in all treatments. Similar abnormalities were reported by Geri et al. (2002) when they evaluate the genotoxic effects of crude extract of Khat leaves after 2000 mg/kg treatment (AL-Zubairi et al., 2008).

Ring chromosomes (Figure 3) induced in a considerable percentage, and is increased linearly with the highest concentration of alkaloids at 48 h exposure time (Table 2). These results are in line with Abderrahman (1998) on studying the effect of Peganum harmala extract on Maize root tips. Ring chromosomes results from stickiness (Evans, 1969) and double strand breakage (lesions), and also id due to exchange type of interaction, which takes place between the two lesions after formation of a looped structure (Bryant, 1998).

Disturbed metaphase and anaphase (Figure 4) were also noted in almost all treatments. The formation of disturbed metaphase and anaphase might be due to a disturbance in the mechanism of chromosome movement (Abo-El-Khier and Abo-ElKhier, 1992). Other types of chromosomal aberrations such as fragments, micro, and micronuclei bridges are also noticed but in very low frequencies. Khat extracts treatment cause an increase in aberrant metaphases. Chromatid gaps were shown to be the most frequent type of aberration followed by chromatid breaks. Similar abnormalities were reported by Geri et al. (2002) while acentric fragments were observed to be less frequent when they evaluate the genotoxic effects of crude extract of Khat leaves after 2000 mg/kg treatment (AL-Zubairi et al., 2008).

Thus, various types of chromosome aberrations were induced by Catha edulis (Khat). The percentage of these abnormalities is increased with the increases of both concentration and exposure time in all treatments. Similar increase in the total percentage of total abnormalities in Vicia faba and Allium cepa after treatment with Vinca alkaloids was reported by Abed EL Tawab (1983). Moreover, the results obtained from this study are in line with Abu El Kheir and Abu El Kheir (1992) on studying the effect of harmole and harmine alkaloids extracted from Peganum harmala on mitosis of Allium cepa.

Mitodepressive effect of Khat on bone marrow of mice has been reported by Omari et al. (1996). Thus, the decrease in mitotic index in higher concentrations might be due to the action of alkaloids on the onset of mitosis which differ from the action of colchicine in its action.

In conclusion, the present study indicates that Catha edulis (Khat) extract probably has some interactions with DNA metabolism in mice, resulting in SCEs and suggesting potential mutagenic effects. Moreover, the

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<th>Exposure (h)</th>
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*%: Total No. of chromosomal aberrations / Total No. of cells examined. Control= 10 µl

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present study suggests that Khat is a potent genotoxic agent. Thus, additional studies under various conditions would be helpful in placing the magnitude of genotoxic and cytotoxic responses to Khat extracts in proper perspective.

References


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