JJBS

Volume 1, Number 4, December. 2008
ISSN 1995-6673
Pages 165 - 172

Jordan Journal of Biological Sciences

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

Salim M. Abderrahman*, Nabeel Modallal

Department of Biological Sciences and Biotechnology. The Hashemite University, P.O. Box 150459. Zarqa 13115, Jordan.

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.

الملخص

يعتبر مضغ القات في المجتمعات الافريقية و جنوب غرب الجزيرة العربية من العادات المتجذره في هذه المناطق مسبباً العديد من المشاكل الاجتماعية و الاقتصادية، و يهدف هذا البحث الى دراسة السمية الوراثية لمستخلص نبات القات حيث تبين ان المستخلص يؤدي الى ارتفاع ذي دلالة احصائية في معدل تبادل الكروماتيدات الشقيقه وان هذه الزيادة تزداد بازدياد تركيز المستخلص، كما أن المستخلص أدى تلى ظهور أنواع مختلفه من أنواع الشذوذ الكروموسومي وتشمل: الكروموسومات الحلقية، الكروموسومات اللزجة، الكروموسومات المتكسره، الكروموسومات المضطربه، و نسبة هذه الانواع تزداد بازدياد كل من تركيز المستخلص والمدة الزمنية، كما ظهرت أنواع اخرى من الشذوذ ولكن بمعدلات متدنية.

Key words: Genotoxicity; Khat; Catha Edulis; Mice Bone Marrow; Chromosomal Aberrations

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

* Corresponding author. salim1954@yahoo.com

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 problems 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 Khatrl , 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 capase-1, capase-3 and capase-8 (Dimba et al. 2004).

The cytological effects of *Catha edulis* in somatic and male germ cells of mice have been demonstrated by Qureshi et al. (1988). They found that Khat significantly increased the frequency of micronucleated polychromatic erythrocytes; and induced bone marrow depression, and reduced the mitotic index of the somatic cells. Khat also induced significant-chromosomal aberration, namely; aneuploids, autosomal univalent, univalent of the sex chromosomes, and polyploids.

Khat consumption leads to formation of miconuclei in human buccal and bladder mucosa (Kassie, 2001). Relatively, little information regarding the genotoxic effects of Khat is available. The genotoxic potential of Khat has been carried out by Tariq et al. (1986) in Swiss albino mice. They studied the effect of Khat during the different stages of spermatogenic cycle and on the rate of pregnancy and post implantation losses. They found that Khat reduced the percent pregnant rates and increased the mean post-implantation losses in treated group. The increase was found to be statistically significant in postmeiotic stages.

There are numerous genotoxic bioassays, but each has its own specific attributes and limitations. Therefore, appropriate bioassays must be used in order to determine the genotoxic properties of Khat.

Numerical chromosome aberrations induced by khat extract in somatic cells of mice have been reported (Qureshi et al. 1988) while blood samples were analyzed for chromosomal aberrations assay by AL-Zubairi et al. (2008).

In view of the above, it is clear that understanding of Khat effect is of a particular interest. For this purpose, the present work is planned in order to investigate the capability of Khat in inducing genotoxic effects on mice genome. Sister chromatid exchange (SCE) and chromosome aberration test are selected and will be employed to achieve this purpose. It is obvious from the literature that genotoxic effects of *Catha edulis* (Khat) extracts in terms of their capacity to induce sister chromatid exchange (SCE) have not been investigated before.

2. Materials and Methods

The dried material of *Catha edulis* (Khat) leaves was grounded 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 intraperitonial 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.

2.1. Sister Chromatid Exchange (SCE):

For each dose, four animals weighing 20-25 g were used .At least 400 somatic bone marrow cells of second metaphase were analyzed. The bromodeoxyuridine tablets were prepared and implanted subcutaneously to Khat treated animals with 10, 20, and 40 mg/kg body weight. Bone-marrow harvest, and slide preparations were performed as described by Allen et al. (1978).

The method of Goto et al. (1978) was used in order to obtain differential staining of sister chromatids. SCE frequencies were counted from the microscope images of the second division cells. An interstitial exchange segment was counted as 2 SCEs. Mitomycin C (MMC) was used as a positive control because of its ability to induce SCEs while dimethylsulfoxide (DMSO) was used as negative control. Students t-test was used to compare the level of significance of the results for the Khat-treated groups and the untreated control as well as the various treated groups.

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).

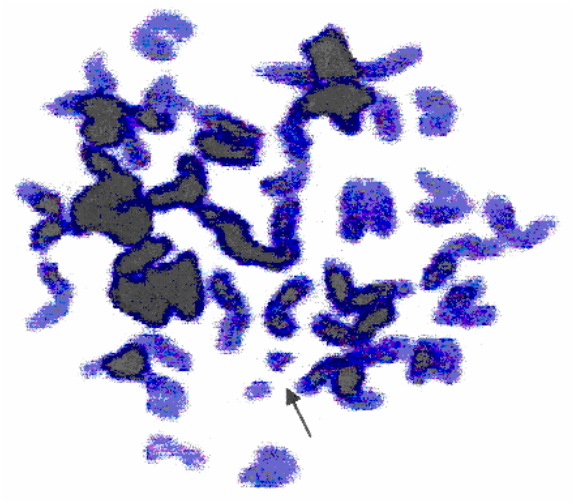

Figure 1. broken chromosomes (arrow) after treatment with 100 mg/kg b.w. aqueous extract from Khat (*Catha edulis)* for 48 hrs. Bar represents 5 µm in length.

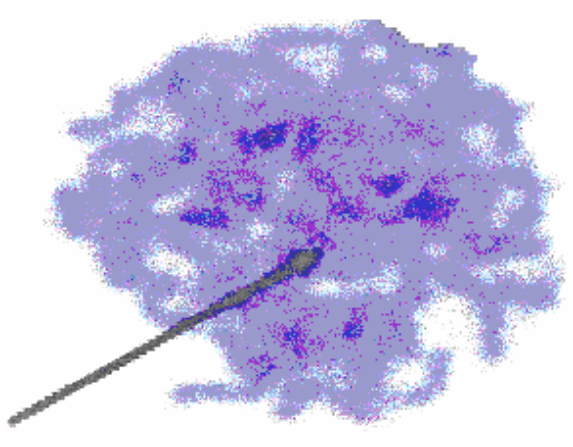

Figure 2. sticky chromosomes (arrow) after treatment with 100 mg/kg b.w. aqueous extract from Khat (*Catha edulis)* for 48 hrs. Bar represents 5 µm inlength.

The ability of Khat extract to induce the formation of chromosomal aberrations was assessed using mouse bone marrow cells. Scanning of the chromosomal aberration concentrated on the followings; broken chromosomes (Figure1), sticky chromosomes (Figure2), ring chromosomes (Figure3), and chromatid disturbances (Figure4). Other types of chromosomal aberrations such as fragments, micro, and macronuclei and bridges were also noticed but in very low frequencies.

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).

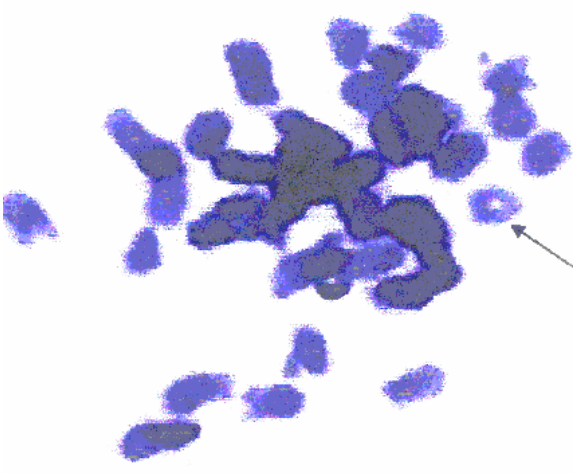

Figure 3. ring chromosomes (arrow) after treatment with 100 mg/kg b.w. aqueous extract from Khat (*Catha edulis)* for 48 hrs. Bar represents 5 µm in length.

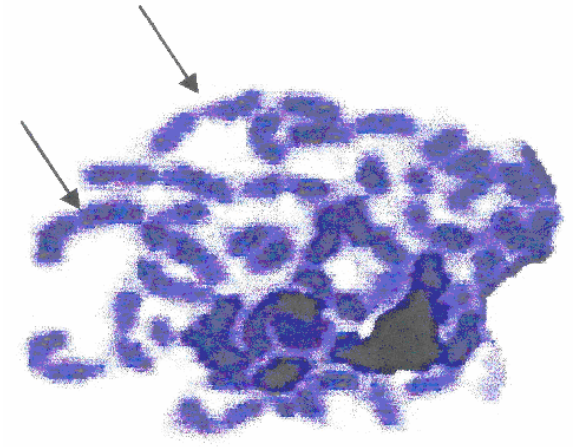

Figure 4. Disturbed metaphase and anaphase (arrows) after treatment with 100 mg/kg b.w. aqueous extract from Khat (*Catha edulis)* for 48 hrs. Bar represents 5 µm in length.

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
	100	5.27	
10	100	5.15	
	100	5.33	
	100	5.56	5.33 ± 0.27*
20	100	5.51	
	100	5.67	
	100	5.50	
	100	5.22	5.48 ± 0. 18*
40	100	5.81	
	100	5.51	
	100	5.80	
	100	5.94	5.77± 0.26*
MMC 2.0	100	10.36	
	100	10.51	
	100	10.55	
	100	10.60	10.55± 0.14
DMSO	100	3.15	
	100	3.47	
	100	3.77	
	100	3.85	3.56± 0.24

*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* (populary 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 akkaloids 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.

Table 2. Chromosomal aberrations *in vivo* bone marrow cells of mice treated with different concentrations of Khat extract for 6, 24 and 48h.

Exposure (h)	Dose (mg/kg) b.w	Cells examined	Aberration types				
			Disturbed metaphase and anaphase	Ring chromosomes	Broken chromosomes	Sticky chromosomes	%*
6	DMSO[a]	4120	-	4	6	-	0.24
	10	4200	-	15	16	-	0.74
	25	4000	1	26	30	15	1.80
	50	4050	2	26	40	30	2.42
	100	4100	6	25	45	51	3.10
24	DMSO[a]	4000	2	7	8	1	0.45
	10	4266	4	13	22	2	0.96
	25	4520	8	26	45	18	2.15
	50	4030	9	35	55	26	3.10
	100	4150	12	42	70	48	4.18
48	DMSO[a]	4000	1	8	9	3	0.53
	10	4008	6	16	23	7	1.30
	25	4222	9	28	48	18	2.44
	50	4175	12	42	60	38	3.64
	100	4250	12	48	71	55	4.38

*%: Total No. of chromosomal aberrations / Total No. of cells examined. Control= 10 µl

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 through time of exposure.

Stickiness has been attributed to an action on the proteins of chromosomes (Badr, 1982), and may be due to the increase in viscosity of the cytoplasm (Abderrhman, 1998). This finding is in line of Al-Meshal (1987) when he tested the effect of cathinone, from *Catha edulis* (Khat) on *Allium cepa* root tips. Cathinone produced significant sticky chromosomes.

Ring chromosomes (Figure 3) induced in a considerable percentage, and is increased linearly to reach a highest percentage 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 (Figure4) 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 macronuclei 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

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

Abdel Tawab S. 1983. Chemical study of *Cantharnthus roseus* cultivated in Egypt and cytological effect of some isolated constituents, MSc. Thesis. Ain Shams Univ. Egypt.

Abderrahman SM.1998. Cytogenetic effects of *Peganum harmala* extract on Maize root tips. Cytologia, 63:283-29 1.

Abo-El-Khier ZA and Abo-El-Khier GM. 1992. Cytological effects of certain active constituents of *Peganurn harmala* L. 1. Effect of Harmol and Harmine alkaloids on mitosis of *Allium cepa*. Journal of King Saud University, 4:37-45.

Ahmad MB and El-Qirbi AB.1993. Biochemical effects of *Catha edulis*, Cathine and Cathinone on adrenocortical function. J. Ethnopharmacol. 39:213-316.

Al Habori M, Al Aghbari A, Al mamary M and Baker M. 2002. Toxicological evaluation of *Catha edulis* (Khat) leaves in long term feeding experiment in animals. J. Ethnopharmacol. 83(3):209-217.

Al Meshal IA. 1987. Mitodepressive effect of cathinone, from *Catha edulis* (Khat), on the meristematic region of *Allium cepa* root tips. Toxicon. 25(4):451-454.

Al Meshal IA, Ageel M, Tariq M and Parmer NS. 1983. The gastric anti-ulcer activity of Khat (*Catha edulis*). Res. Comm. Subs. Abuse 4: 143-149.

Al-Ahdal MN, McGarry TJ and Hannan M. 1988. Cytotoxicity of Khat (*Catha edulis*) extract on cultured mammalian cells. Mutat. Res. 204:3 17-322.

Allen JW, Shuler CF and Dan SA. 1978. Bromodeoxyuridine tablet methodology for *in vivo* studies of DNA synthesis. Somatic Cell Genetics. 4: 393-405.

Al-Mamary M, Al-Habori M, Al-Aghbari AM and Baker MM. 2002. Investigation into the toxicological effects of *Catha edulis* leaves: a short term study in animals. Phytother. Res.16: 127-132.

Al Meshal IA, Tariq M, Parmer, NS and Ageel M. 1985. Anti-inflammatory activity of the flavonoid fraction of Khat (*Catha edulis*). Agents and Action.17:3-4.

Al-Meshal I, Qureshi S, Ageel AM, Tariq M. 1991. The toxicity of *Catha edulis* in mice. J. Subst. Abuse. 3:107-115.

AL-Zubairi A, Ismail P, Pei CP and Rhmat A. 2008. Genotoxic effect of *Catha edulis* (Khat) crude extract after sub-chronic administration in rats. Env. Tox. Pharm. 25:298-303.

Ayoub MT, Rashan RJ, Khazraji AT and Adday MH. 1989. New oxiamide from *Peganum harmala* seeds. Phytochemistry 28:2000.

Badr A. 1982. Cytogenetic activities of 3 sulphonamides. Mutat. Res. 104: 95-100.

Balint GA, Ghebrekidan H and Balint EE. 1991. *Catha edulis* and international sociomedical problem with considerable pharmacological implications. East Afr. Med. J. 68:555-561.

Bruckman E, Wojcik A and Obe G. 1999. X-irradiation of G1 CHO cells induces SCE. Chromosome Res.7:277-28.

Bryant PE. 1998. Mechanisms of Radiation-induced chromatid breaks. Mutat. Res. 404:107-111.

Carvalho F. 2003.The toxicological potential of Khat. J. Ethnopharmacol. 87 (1):1-2.

Chatterjee A and Deb S.1999. Genotoxic effect of arecoline given either by the peritoneal or oral route in murine bone marrow cells and the influence of N-acetylcysteine. Cancer Lett. 139(1): 23-31.

Culvenor CC, Dann AT and Dick AT. 1962. Alkylation as the mechanism by which the hepatotoxic pyrrizidine alkaloids on cell nuclei. Nature 195: 570.

Das A, Mukherjee A and Chakrabarti J. 2004. Sanguinarine: an evaluation of in cytogentic activity. Mutat Res. 563(1): 81-7.

Dhadphale, M and Omolo, OE. 1988. Psychiatric morbidity among Khat chewers. East. Afr. Med. J. 65:355-359.

Dimba E, Gjertsen BT, Bredholt T, Fossan KO, Costea DE, Francis GW, Johannessen AC and Vintermyr OK. 2004. Khat (*Catha edulis*)-induced apoptosis is inhibited by antagonists of capase-1 and -8 in human leukemia cells. Br. J. Cancer 91, 1726

Dimba E, Gjertsen BT, Francis GW, Johannessen AC and Vintermyr OK.2003. *Catha edulis* (Khat) induces cell death by apoptosis in human leukemia cell lines. Ann. N. Y. Acad. Sci. 1010, 384

Dimba E, Gjertsen BT, Francis GW, Johannessen AC and Vintermyr OK. 2003. *Catha edulis* (Khat) induces cell death by apoptosis in leukemia cell lines.Ann. N.Y. Acad. Sci.1010:384-388.

Elhag H, Mossa JS and El Olemy, MM. 1999. Antimicrobial and cytotoxic activity of the extracts of Khat callus cultures. In : J. Janick (ed), Perspectives on new crops and new uses. ASHS press, Alexandria, A.

Eriksson M, Ghani NA and Kristiansson B. 1991. Khat -chewing during pregnancy: effect upon offspring and some characteristics of the chewers. East. Afr. Med. J. 68:106-111

Evans HJ. 1969. The induction of chromosome aberrations by N-mustard and its dependence on DNA synthesis. Proc. Roy. Soc. Ser. B. Biology 173:491-512.

Granek M., Shalev A and Weingerten A.M. 1988. Khat induced hypnegogic hallucinations. Acta Psychiatr. Scand. 78: 458-461.

Giannini AJ and Castellani S. 1982. Amanic-like psychosis due to Khat (*Catha edulis* Forsk). J. Toxicol. C1in. Toxicol. 19:455-459.

Giri S., Giri A., Sharma G.D and Prasad S.B. (2002). Mutagenic effects of carbosulfan, a carbamte pesticide. Mutt. Res. 519: 75-82.

Goto KS, Maeda S, Kano Y and Sugiyama T. 1978. Factors involved in differential Giemsa-staining of sister chromatids. Chromosoma, 66:351-359.

Hannan MA, Aboul-Enein HY and Al-Dakan AA. 1985. Histidine reversion in Ames *Salmonella* strains induced by extracts of Khat, A substance of abuse. Res. Comm. Subs. Abuse. 6:179 -187.

Kabarity A and Malallah G. 1980. Mitodepressive effect of Khat extract in the meristematic region of *Allium cepa* root tips. Cytologia 45:733-738.

Kabarity A, E1-bayoumi A and Habib AA. 1979. Mitodepressive effect and stathmokinetic action of pantopon hydrochloride. Mutat. Res. 66:143-148.

Kalix P and Khan I. 1984. An amphetarnine-like plant material. Bull. WHO, 62: 681-686.

Kalix P. 1990. Pharmacological properties of the stimulant Khat. Pharmacol. Ther. 48:397-416.

Kalix P. 1991. The pharmacology of psychoactive alkaloids from ephedra and catha. J. Ethnopharmacol. 32:201-208.

Kassie F, Darroudi M, Schulte-Hermann R, Knasmuller S. 2001. Khat (*Catha edulis*) consumption causes genotoxic effects in humans. Int. J. Cancer 92:329-332.

Khalil A. 1996. The *in vitro* genotoxic activity of Albendazole and Praziquantel, singly and in combination on human blood lymphocytes. Qatar Univ. Sci. J. 16 (1): 103-108.

Mclaren P. 1987. Khat-induced paranoid psychosis (Letter). Br. J. Psychiatry. 150:875-876.

Mwenda JM, Arimi MM, Kyama MC and Langat DK. 2003. Effects of Khat (*Catha edulis*) consumption on reproductive functions: a review. East Afr. Med. J. 80(6):318-323.

Nasr AH, Khatri ML. 2000. Head and neck squamous cell carcinoma in Hajjah, Yemen. Saudi Med. J. 21(6):565- 568.

Nencini P and Ahmed AM.1989. Khat consumption: a pharmacological review. Drug Alcohol Depend. 23:19-29.

Nencini P, Ahmed AM and Elmi AS. 1986. Subjective effects of Khat chewing in humans. Drug Alcohol Depend. 18:97-105.

Omari YI, Shraidah Z, Abderrahman SM. 1996. Mitodepressive effect of Khat (*Catha edulis*) on the bone marrow of mice. Biomed. Lett. 54:69-72.

Osborne C.1983. The curse of north Yemen, Nursing Mirror, 156:24- 28.

Palitti F. 1998. Mechanisms of the origin of chromosomal aberrations. Mutat. Res. 20:403-416.

Peter PE, Xia Q, Lin G and Ming WC. 2002. Genotoxic pyrrolizidine alkaloids-Mechanisms leading to DNA adduct formation and tumorigenicity. Int. J. Mol. Sci. 3:945-961.

Qureshi S, Tariq M, Parmar NS and Al Meshal.1988. Cytological effects of Khat (*Catha edulis*) in somatic and male germ cells of mice. Drug.Chem.Toxicol. 11(2): 151-165.

Riebeiro LR, Silva AR, Bautista AR, Costa SL, Sales LA, Rois AC and Salvadori DM. 1993.Clastogenic effect of extracts obtained from *Crotalaria retusa* L. and Crotalaria mucronata Desv. on mouse bone marrow cells. Mutat. Re. 300:253-258.

Soufi HE, Kameswaran M and Malatani T. 1991.Khat and oral cancer. J. Laryng. Otol. 105:643-645.

Tariq M, Al Meshal IA, Pamar, NS, Ageel AM and Qureshi S.1986. Evaluation of genotoxic potential of Khat (*Catha edulis*) in Swiss albino mice. Mutagenesis. 1 (5): 381-382.

Tariq M, Pamar NS, Qureshi, S, El-Feraly FS and Al Meshal IA. 1987. Clastogenic evaluation of cathinone and amphetamine in some cells of mice. Mutat. Res.190 (2):153- 157.

Tavares DC and Takahashi CS. 1994. Evaluation of the genotoxic potential of the alkaloid boldine in mammalian cell systems in vitro and *in vivo*. Mutat. Res. 321:139-145.

Toennes SW, Harder S, Schramm M, Niess C and Kauert GF. 2003. Pharmacokinetics of cathinone, cathine and norephedrine after chewing Khat leaves. Br. J. Clin. Pharmacol. 56: 125-130.

Tucker JD, Auletta A, Cimino MC, Dearfield KL, Jacobson-Kram D and Carrano AV. 1993. Sister chromatid exchange: second report of the Gene.Tox. Program. Mutat. Res. 297: 101-180.

Widler P, Mathys K, Brenneisen R, Kalix P and Fisch, HU. 1994. Pharmacodynamics and pharmacokinetics of Khat: a controlled study. Clin. Pharmacol. Ther. 55:556-562