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Genotoxicity Assessment of Fresh Khat Leaves Extract in Chinese Hamster Ovary Cell Lines

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Due to its stimulating and pleasurable effects, chewing the leaves of khat (*Catha edulis*) is a widespread habit among the people of East Africa and Arabian Peninsula. The aim of this study was to assess the genotoxicity of the fresh khat leaves extract in CHO cell lines using two cytogenetic endpoints, Chromosomal aberrations (CAs) assay and Micronucleus test (MN). The chromosome aberrations and micronucleus induction test were performed without any metabolic activation system. Data were analyzed by Chi-square using SPSS version 24.0. High concentrations of fresh khat leaves extract (500 and 5000 $\mu\text{g mL}^{-1}$) were found to reduce the mitotic index (MI) significantly ($p < 0.05$), while only the highest concentration (5000 $\mu\text{g mL}^{-1}$) was found to induce chromosomal aberrations. In contrast, the results of MN induction test showed no significant micronuclei formation after treatment with different concentrations, however, the cytochalasin-B proliferation index (CBPI) was found to be affected by the highest concentration of the fresh khat leaves extract. Only high concentrations of fresh khat leaves extract induced chromosomal aberrations and dicentric, rings chromosomes, breaks and exchanges were the main types of aberrations induced by fresh khat leaves extract.

Key words: Khat, genotoxicity, chromosome aberrations, micronucleus, Chinese hamster ovary cell lines

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INTRODUCTION

Chewing khat habit represents a major socio-economic problem in the Southern Arabia and many countries of the Horn of Africa. The leaves of *Catha edulis* (*Celastraceae*) (khat) are chewed for its sympathomimetic and stimulating effects. Khat leaves are chewed due to their pleasurable and stimulating effects. It is chewed mainly in countries of Eastern Africa regions and Southern Arabia¹⁻⁴. The use of khat has spread worldwide, however, it has until recently remained mostly confined to the regions where the plant is planted since only fresh leaves have the potency (of cathinone) to produce the desired pleasurable and stimulating effects. Due to its structural similarity with amphetamine, cathinone is the most important active ingredient of khat, which causes the major pharmacological effects⁵. Other constituents of khat leave include tannins, amino acids and a significant amount of ascorbic acid, magnesium and beta-carotene^{1,6}. The market value of the leaves correlates with their cathinone contents¹. The detrimental and common adverse effects of the active principle of khat on man and animals include analgesia, insomnia, anorexia, irritability, hyperthermia, mydriasis and endocrinological disturbances have been described⁷⁻⁹. Acute autonomic responses, such as elevated blood pressure and tachycardia, have been reported^{10,11}. The chronic use of khat has also been associated with the development of hypertension¹².

The major compounds are absorbed in the oral cavity due to the efficient alkaloid extraction by chewing¹³. Increase in micronucleated buccal cells was observed in khat chewers¹⁴. Leaves extract administration was found to inhibit RNA and DNA synthesis in the neurons of chick embryo¹⁵ and reduce DNA and RNA contents in liver and brain tissue homogenates in rat¹⁶. Meanwhile, cytotoxicity and mutagenicity to mammalian cells have been reported after treatment with methanolic khat leaves extract¹⁷. Numerical chromosomal aberrations have been reported to be induced by khat extract in somatic cells of mice¹⁸ while structural chromosomal aberrations in rat blood samples were observed by Al-Zubairi *et al.*¹⁹. The cytotoxic effects of whole khat extract on three leukemia cell lines (HL-60, Jurkat and NB4 cells) were reported²⁰ and apoptosis induction through a mechanism involving activation of caspase-1, caspase-3 and caspase-8²¹. Cytotoxic and genotoxic effects on human cell lines CEM has also been reported²².

This study reported the results obtained on the genotoxic effects of fresh khat leaves extract in Chinese hamster ovary (CHO) cell lines, using chromosomal aberrations assay (CAs) and micronucleus (MN) formation as cytogenetic endpoints. This study described the results of those investigations and comments on the *in vitro* genotoxic effects of fresh khat leaves extract in CHO cell line.

MATERIALS AND METHODS

Khat extract: Fresh khat leaves have been obtained from khat sellers in Kuala Lumpur. Khat leaves have been washed thoroughly, blended with juicer and the leaves juice has been filtered and used immediately for cell cultures treatment. Khat leaves extract doses were expressed according to starting fresh plant material weight.

Chromosomal aberration assay: In genotoxicity testing, the potential of a chemical to induce chromosomal damage is usually investigated. Therefore, the fresh khat leaves extract was evaluated for its potential to induce chromosome aberrations and micronuclei formation in CHO cell lines.

CHO cell lines: Chinese hamster ovary (CHO) cell lines were purchased from ECACC (UK). Atypical diploid CHO cell contains 21 chromosomes. The cells grow as an adherent monolayer in the tissue culture flask, doubling approximately every 12 h. Cultures were monitored for their health status throughout the experiment by cell counting and microscopic observation. Culture medium used to maintain the cell lines was RPMI 1640 medium (PAA Laboratories GmbH, Germany) supplemented with 10% foetal bovine serum (PAA Laboratories GmbH, Germany) using sterile Nunc tissue culture flasks (Nunc, Denmark). The cells were incubated in a humidified tissue culture incubator at 37°C and 5% CO₂. Cells were trypsinized with 0.05% trypsin (PAA Laboratories GmbH, Germany) when reach approximately 60-80% confluency, collected by centrifugation and seeded in fresh medium. Each trypsinization step was recorded as the cells have one passage. A continued culture of CHO cell line (passage 15) was used. On the day before the experiment, 5×10⁵ cells from a culture with approximately 60-80% confluence were seeded into each T-25 cm² (Nunc, Denmark) flasks. The cells were incubated overnight in a humidified incubator at 37°C and 5% CO₂.

Treatment with fresh khat leaves extract: Fresh khat leaves extract was prepared as 1 mg mL⁻¹ stock solution in absolute ethanol just prior to use and a dosing volume of stock concentration diluted with medium ensured a final ethanol concentration of <1%. Overnight cell cultures were examined under an inverted microscope and appeared healthy, well adherent, evenly distributed and 60-80% confluent. For each extract concentration and controls, duplicate cultures were prepared. Control cultures were handled in the same manner to the treated cultures. Mitomycin-C (Sigma, Germany) was used as a positive control. The treatment medium was 5 mL of the cell culture medium with 10% foetal bovine serum, with the treatment concentration or a control solution and the final concentrations were 0, 5, 50, 500 and 5000 µg mL⁻¹. Cells were cultured in the treatment medium for 24 h.

Cells Harvest and Preparation of chromosome slides: Cells for chromosomal aberration assay were harvested at the end of treatment. Colcemid (PAA Laboratories GmbH, Germany) was added 2 h prior to harvesting, at 0.2 mL to arrest cells in metaphase. Then the medium with suspended cells was transferred into centrifuge tubes. The attached cell monolayer was dissociated with 0.05% trypsin and pooled with the collected cell suspension. After that, the cells were centrifuged at 800 rpm and resuspended in phosphate buffered saline (Sigma), collected, swelled in 0.075 M KCl (Sigma) and fixed in Carny's fixative (3:1 mixture of methanol and glacial acetic acid) for 3 times. Next, the tubes were centrifuged for 5 min, the supernatant discarded and the cell suspension dropped onto pre-chilled slides previously cleaned with non-toxic detergent and soaked in distilled water at 5°C. Air dried chromosome slides were prepared and stained in 6% Giemsa stain ([CAS 67-56-1] Gibco, Invitrogen, Germany). One thousand cells were examined for each duplicate culture to score mitotic index. The mitotic index (MI) was calculated as the percent of cells at the mitotic stage. Relative mitotic index (RMI) was calculated as Baak²³:

$$\text{RMI (\%)} = \frac{\text{Test concentration MI}}{\text{Solvent control MI}} \times 100$$

Analysis of chromosome aberrations: All slides were randomly coded and well-spread metaphase cells with 19-23 chromosomes were analyzed for chromosome aberrations as defined. Two hundred metaphase cells from each negative control, positive control and fresh khat leaves extract-treated cultures were scored. The number of each type of aberration and the percent of cells with aberrations were recorded and summarized. The number of chromatid gaps and chromosome gaps were recorded when encountered, but not included in the calculations²⁴. The percentages of cells with aberrations from each concentration were compared to the solvent control values using χ^2 analyses.

Micronucleus test (MN)

Cell culture treatment: The CHO cell cultures were prepared as previously described in the chromosomal aberrations assay. After 22-24 h the media was removed and the cells were washed with PBS or media and fresh media containing 6 $\mu\text{g mL}^{-1}$ Cytochalasin-B (CB [CAS 4930-96-2], Sigma-Aldrich, Germany) was added and incubated for further 22-24 h. One set of negative and positive controls were run for each experimental day.

Harvest and slide preparation: To examine binucleated cells for micronuclei *in vitro*, the technique utilized was that described by Fenech²⁵. After completing treatment, the cells

were trypsinized and suspended in pre-warmed hypotonic solution (0.075 M KCL) and carefully homogenized with a Pasteur pipette. Then the cell suspension was centrifuged again and re-suspended in pre-chilled 10 mL of Carney's fixative, methanol/acetic acid (3:1 v/v), centrifuged and dropped onto pre-chilled slides. The slides were stained with 6% Giemsa diluted in phosphate buffer (pH 6.8) for 10 min, washed with distilled water, air-dried and examined under the microscope.

Scoring: The MN induction was determined in 1000 binucleated cells with the cytoplasm well preserved and clearly surrounded with nuclear membrane, having an area of less than one third of that of the main nucleus²⁶. Using a Nikon microscope, cells containing 1 micronucleus were scored blindly. For identification of MN, the criterion used was according to Fenech²⁷. In each treatment, for cell cycle kinetic analysis, numbers of mononucleated, binucleated and polynucleated cells per 1000 cells were counted, the nuclear division index or cytochalasin B proliferation index (CBPI) is determined in a blind test. Cells with well preserved cytoplasm, containing 1-4 nuclei, were scored. The CBPI was calculated according to OECD guideline number 487²⁸ using the following formula:

$$\text{CBPI} = \frac{\text{MI} + 2\text{MII} + 3\text{MIII} + 4\text{MIV}}{\text{N}}$$

A minimum of 200 cells per concentration were analysed (N).

Statistical analysis: Data were analyzed for Chi-square analysis using SPSS version 24.0. All statistical tests were performed at the $p < 0.05$ level of significance.

RESULTS

Mitotic index analysis: A two-fold series of 5 concentrations of fresh khat leaves extract were used. The relative mitotic index (RMI) values (Table 1) revealed an increasing inhibitory effect with increasing concentrations of the khat extract as well as for the positive control MMC. The mitotic index of the two higher concentrations was found to be significantly ($p < 0.001$, Table 1). In contrast, the mitotic index of the two lowest concentrations was observed to be non-significantly reduced compared to the control.

Chromosome aberrations (CA) assay: The CA assay, clastogenicity determined was not significantly observed in the untreated control or the solvent treated control. Positive

Table 1: Mitotic index and relative mitotic index (RMI) observed for CHO cultures treated with different concentrations of Khat extract and controls

Treatments	Concentrations	Number of dividing cells	Total cells counted	Mitotic index (%)	RMI
Khat Extract ($\mu\text{g mL}^{-1}$)	0	59	1000	5.9	100.0
	5	41	1000	4.1	69.5
	50	61	1000	6.1	103.0
	500	33	1000	3.3*	56.0*
	5000	32	1000	3.2*	54.3*
MMC ($\mu\text{g mL}^{-1}$)	1.0	30	1000	3.0*	50.9*

*Significantly different from the control $p < 0.05$

Table 2: Sum of the frequencies of chromosome aberrations (CAs) induced in CHO cultures treated with different concentrations of khat extract and the positive and negative controls

Treatments	Concentrations	Total metaphase	Aberrant metaphase	Chromosome aberrations							Percentage of cell with aberrations
				G	B	A	E	R	DC	End	
Khat extract ($\mu\text{g mL}^{-1}$)	0	200	20	3	0	1	1	6	9	0	10.00
	5	200	14	0	1	0	1	6	5	1	7.00
	50	200	24	3	2	0	1	5	11	2	12.00
	500	200	26	0	3	0	3	1	14	4	13.00
	5000	200	36*	3	4	0	7	3	13	5	18.00
MMC ($\mu\text{g mL}^{-1}$)	1.0	150	71*	2	49	0	12	5	3	0	47.33

G: Gap, B: Breaks, A: Acentrics, E: Chromatide exchange, R: Chromosome ring, DC: Dicentric, END: Endoreduplication. *Significantly different from the control $p < 0.05$

Table 3: Frequencies of micronucleus (MN) formation and cell cycle kinetics on CHO cultures treated with different concentrations of Khat extract together with MMC treated and untreated cultures

Treatments	Concentrations	BN	MNi	MNi (%)	Cell cycle kinetics ^a				
					M1	M2	M3	BN (%)	CBPI
Khat extract ($\mu\text{g mL}^{-1}$)	0	1000	22.0	2.20	54	120	26.00	60.00	1.860
	5	1000	20.0	2.00	46	126	28.00	63.80	1.910
	50	1000	15	1.5	56.00	129	25	64.50	1.945
	500	1000	20	2.0	56.00	111	33	55.50	1.885
	5000	1000	25	2.5	145.00	47	8	23.56	1.315*
MMC ($\mu\text{g mL}^{-1}$)	1.0	1000	55.0*	5.50	143	46	11.00	23.00	1.340*

^aThe numbers of mononucleated (M1), binucleated (M2) and polynucleated (M3) cell per 200 cells were quantitated for cell cycle kinetic analysis. *Significantly different from control $p < 0.05$

control (MMC) was significantly shown to induce chromosome aberrations. On the other hand, the clastogenic indices of the highest concentration of fresh khat leaves extract was found to induce chromosome aberrations significantly ($p < 0.01$) as shown in Table 2. Gaps, breaks as well as exchanges, endoreduplications, ring chromosomes and dicentrics were the main types of aberration observed. In treated cultures of CHO cell line the number of breaks was found to be significantly increased in the highest concentration of the khat leaves extract. However, ring chromosomes and dicentrics were found to be non-significantly increased in all treated cultures. Meanwhile, endoreduplications were found to be high in the two highest concentrations. The number of aberrant cells with structural aberrations including or excluding gaps recorded in cultures treated with the highest concentrations ($5000 \mu\text{g mL}^{-1}$) increased significantly $p < 0.01$ (Table 2) when compared with the untreated groups. Fisher's exact test was used to test the statistical significance of the CA results.

MN induction: The potential of fresh khat leaves extract to induce micronucleus is shown in Table 3. Treatment of CHO cells with khat leaves extract was to have no effects in the MN induction test. In the cell cycle kinetics analysis or CBPI, treatment with the highest khat extract concentrations ($5000 \mu\text{g mL}^{-1}$) was found to exert an inhibition of cell proliferation in CHO cells (Table 3), while other concentrations were found to be reduced non-significantly compared to the negative control treated cells.

DISCUSSION

People of Southern Arabia and East Africa habitually chew khat leaves, which produce amphetamine-like stimulating effects. Habitual khat chewing results in social and public health problems among these populations. It has been reported that khat leaves contain 14.5% tannins, mainly in the form of condensed type tannins²⁹ and other polyphenolic and flavonoid compounds. The mutagenicity of *C. edulis* leaves

extract has been studied previously in bacteria by Hannan *et al.*³⁰ and rats by Al-Zubairi *et al.*¹⁹. As well as by De Hondt *et al.*¹⁶, who studied the acute effect of khat administration and observed the chromosomal aberrations formation after subcutaneous treatment of rats with methanolic khat extract. In addition, Tariq *et al.*³¹ studied the prolonged exposure of mice to khat extract and reported khat extract to induce total and partial sterility in male and post-implantation loss in female and reduced the percent pregnancy rates. Khat extract was also reported to increase the frequency of micronucleated polychromatic erythrocytes, induced bone marrow depression and reduce the mitotic index of the somatic cells¹⁸, as well as the sister chromatid exchange and various chromosomal aberrations in mice bone marrow cells³².

It was found that drying of khat leaves results in loss of some active components like cathinone, therefore, people chewing khat usually prefer to use the green fresh leaves¹. In this study it was tried to evaluate for the first time, the genotoxic effects of fresh khat leaves extract in CHO cell lines. This investigation was conducted using the chromosomal aberration assay and micronucleus test as cytogenetic end-points. Fresh khat leaves extract reduced the mitotic index at high extract concentrations and induced higher chromosomal aberrations frequencies as compared to control values as revealed by the CA assay. The percentage of structurally damaged cells in the MMC (positive control) treatment group was statistically significantly increased compared to the solvent control indicating the responsiveness of the cells in this test system. Genotoxic agents have the potential to interact with DNA and may cause DNA damage. Chromosomal aberrations occur in proliferating cells and are regarded as a manifestation of damage to the genome. Chromosomal aberrations assay has been commonly used as a test of genotoxicity in order to evaluate cytogenetic responses to chemical exposure. The results found, at least under the experimental conditions used in the present work, showed the ability of this extract at high concentration, to induce genotoxicity and cytotoxicity *in vitro* in CHO cells. These results were found to be in agreement with that reported by Barkwan *et al.*²², who studied the cytotoxic and genotoxic effects of khat extract on human cell lines CEM.

Formation of dicentric and ring chromosomes may suggest telomeric loss or telomeric associations as well as chromatid/chromosome breaks that starts chromosomal breakage-fusion-bridge cycle and finally results in the production of mitotically unstable chromosome aberration, i.e., ring chromosome, dicentric chromosome or telomeric association in metaphase cells^{33,34}. Endoreduplication in eukaryotic cell is a process that involves DNA amplification without cell division^{35,36}. Inhibition of DNA topoisomerase II activity results in the formation of endoreduplication^{35,37,38} and increase in the number of endoreduplicated cell chromosomes may indicate that the test substance is capable of inhibiting cell cycle progression.

In addition to chromosomal aberration assay, MN induction test is another widely used cytogenetic method to assess *in vitro* clastogenic potential of substances. The frequency of occurrence of micronuclei in treated cells provides a comparatively rapid and sensitive indication of both chromosomal aberrations and loss that lead to numerical chromosomal anomalies^{39,40}. Micronuclei are chromatin masses formed in the cytoplasm with the appearance of small nuclei result from chromosome fragments at anaphase or from acentric chromosomal fragments. Induction of micronuclei provides a quantifiable measure of recent DNA damage result from acentric fragments or whole chromosomes fragment as a result of an exposure to either clastogenic or an aneuploidogenic effect^{41,42}. The present study failed to show significant effects of exposure of CHO cells to fresh khat leaves extract. However, there were no significant changes in the induction of micronuclei formation between the treated cultures and the negative control cultures. Meanwhile the cell cycle kinetics represented by the CBPI showed that the highest concentration (5000 µg mL⁻¹) of khat leaves extract reduced the CBPI significantly compared to the negative control. This later result corresponds with that of the chromosomal aberration assay, where the highest concentrations of the fresh khat leaves extract induced chromosomal aberrations significantly.

CONCLUSION

The present results provide evidence that fresh khat leaves extract in high concentrations have a genotoxic effects on cultured Chinese hamster cell line, bringing into highlight the need for further studies to better understand the molecular mechanisms of action of khat leaves extract for a better comprehension. Thus results found, at least under the experimental conditions used in the present work, showed the ability of fresh khat leaves extract to induce genotoxicity *in vitro* in CHO cells.

SIGNIFICANCE STATEMENTS

This study highlights for the first time, the effect of unprocessed fresh khat leaves extract on the chromosomes of the normal cell lines, CHO cell lines, using the two cytogenetic end points chromosomal aberrations assay and micronucleus test. The results of this study may shed a light on the genotoxicity of the fresh extract of the khat leaves.

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REFERENCES

- Hassan, N.A.G.M., A.A. Gunaid and I.M.M. Lyon, 2007. Khat (*Catha edulis*): Health aspects of khat chewing. Eastern Mediterr. Health J., 13: 706-718.
- Kalix, P., 1994. Khat, an amphetamine-like stimulant. J. Psychoact. Drugs, 26: 69 -74.
- Alem, A., D. Kebede and G. Kullgren, 1999. The prevalence and socio-demographic correlates of khat chewing in Butajira, Ethiopia. Acta Psychiatr. Scand., 100: 84-91.
- Belew, M., D. Kebede, M. Kassaye and F. Enquoselassie, 2000. The magnitude of khat use and its association with health, nutrition and socio-economic status. Ethiop. Med. J., 38: 11-26.
- Luscher, C., 1995. Drugs of Abuse. In: Basic and Clinical Pharmacology, Katzung, B.G. (Ed.). 6th Edn., Prentice-Hall, Englewood Cliffs, NJ., USA., ISBN-13: 9780838506196, pp: 482.
- Wabe, N.T., 2011. Chemistry, pharmacology and toxicology of khat (*Catha edulis* Forsk): A review. Addict. Health, 3: 137-149.
- Connor, J., E. Makonnen and A. Rostom, 2000. Comparison of analgesic effects of khat (*Catha edulis* Forsk) extract, D-amphetamine and ibuprofen in mice. J. Pharm. Pharmacol., 52: 107-110.
- Al-Hebshi, N. and N. Skaug, 2005. Khat (*Catha edulis*)-an updated review. Addict. Biol., 10: 299-307.
- Brenneisen, R., H.U. Fisch, U. Koelbing, S. Geissshusler and P. Kalix, 1990. Amphetamine-like effects in humans of the khat alkaloid cathinone. Br. J. Clin. Pharmacol., 30: 825-828.
- Duke, J.A., 1985. CRC Handbook of Medicinal Herbs. CRC Press, Boca Raton, FL., USA., pp: 104.
- Wilder, P., K. Mathys, R. Brenneisen, P. Kalix and H.U. Fisch, 1994. Pharmacodynamics and pharmacokinetics of khat: A controlled study. Clin. Pharmacol. Ther., 55: 556-562.
- Al-Motarreb, A., K. Baker and K.J. Broadley, 2002. Khat: Pharmacological and medical aspects and its social use in Yemen. Photother. Res., 16: 403-413.
- Toennes, S.W., S. Harder, M. Schramm, C. Niess and G.F. Kauert, 2003. Pharmacokinetics of cathinone, cathine and norephedrine after the chewing of khat leaves. Br. J. Clin. Pharmacol., 56: 125-130.
- Kassie, F., F. Darroudi, M. Kundi, R. Schulte-Hermann and S. Knasmüller, 2001. Khat (*Catha edulis*) consumption causes genotoxic effects in humans. Int. J. Cancer, 92 : 329-332.
- Hammouda, M.E., 1971. Effect of khat extract, D-cycloserine and thalidomide on ribonucleic acid of the neurons of 7 and 19 days chick embryo. Bull. Fac. Sci. Riyadh Univ., 3: 64-64.
- De Hondt, H.A., A.M. Fahmy and S.A. Abdelbaset, 1984. Chromosomal and biochemical studies on the effect of Kat extract on laboratory rats. Environ. Mutagen., 6: 851-860.
- Al-Ahdal, M.N., T.J. McGarry and M.A. Hannan, 1988. Cytotoxicity of Khat (*Catha edulis*) extract on cultured mammalian cells: Effects on macromolecule biosynthesis. Mutat. Res./Genet. Toxicol., 204: 317-322.
- Qureshi, S., M. Tariq, N.S. Parmar and I.A. Al-Meshal, 1988. Cytological effects of Khat (*Catha edulis*) in somatic and male germ cells of mice. Drug Chem. Toxicol., 11: 151-165.
- Al-Zubairi, A., P. Ismail, C.P. Pei and A. Rahmat, 2008. Genotoxic effect of *Catha edulis* (khat) crude extract after sub-chronic administration in rats. Environ. Toxicol. Pharmacol., 25: 298-303.
- Dimba, E., B.T. Gjertsen, G.W. Francis, A.C. Johannessen and O.K. Vintermyr, 2003. *Catha edulis* (Khat) induces cell death by apoptosis in leukemia cell lines. Ann. N. Y. Acad. Sci., 1010: 384-388.
- Dimba, E.A.O., B.T. Gjertsen, T. Bredholt, K.O. Fossan and D.E. Costea *et al.*, 2004. Khat (*Catha edulis*)-induced apoptosis is inhibited by antagonists of caspase-1 and -8 in human leukaemia cells. Br. J. Cancer, 91: 1726-1734.
- Barkwan, S.S., C.R. Barnett, Y.A. Barnett, P.T. Tomkins and C.N. Fokunang, 2004. Evaluation of the cytotoxic and genotoxic potential of Khat (*Catha edulis* Forsk) extracts on human T lymphoblastoid cell line. J. Med. Sci., 4: 110-114.
- Baak, J.P.A., 1990. Mitosis counting in tumors. Hum. Pathol., 21: 683-685.
- Swierenga, S.H.H., J.A. Heddle, E.A. Sigal, J.P.W. Gilman, R.L. Brillinger, G.R. Douglas and E.R. Nestmann, 1991. Recommended protocols based on a survey of current practice in genotoxicity testing laboratories, IV. Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures. Mutat. Res./Fundam. Mol. Mech. Mutagen., 246: 301-322.
- Fenech, M., 2000. The *in vitro* micronucleus technique. Mutat. Res./Fundam. Mol. Mech. Mutagen., 455: 81-95.
- Kirsch-Volders, M. and M. Fenech, 2001. Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes. Mutagenesis, 16: 51-58.
- Fenech, M., 1993. The cytokinesis-block micronucleus technique: A detailed description of the method and its application to genotoxicity studies in human populations. Mutat. Res./Fundam. Mol. Mech. Mutagen., 285: 35-44.

28. OECD., 2016. *In vitro* mammalian chromosome aberration test. OECD Guideline for the Testing of Chemicals, Section 4, Test No. 473, OECD, Paris, France, July 29, 2016.
29. El Sissi, H.I. and M.A. Alla, 1966. Polyphenoligs of the leaves of *Catha edulis* (Part I). *Planta Med.*, 14: 76-83.
30. Hannan, M.A., H.Y. Aboul-Enein and A.A. Al-Dakan, 1985. Histidine reversion in Ame's *Salmonella* strains induced by extracts of Khat-A substance of abuse. *Res. Commun. Subst. Abuse*, 6: 179-187.
31. Tariq, M., I.A. Al-Mesheal, N.S. Parmars, A.M. Ageel and S. Qureshi, 1986. Evaluation of genotoxic potential of khat (*Catha edulis*) in Swiss Albino mice. *Mutagenesis*, 5: 381-382.
32. Abderrahman, S.M. and N. Modallal, 2008. Genotoxic effects of *Catha edulis* (khat) extract on mice bone marrow cells. *Jordan J. Biol. Sci.*, 1: 165-171.
33. Gisselsson, D., L. Pattersson, M. Hoglund, M. Heidenblad and L. Gorunova *et al.*, 2000. Chromosomal breakage-fusion-bridge events cause genetic intratumor heterogeneity. *Proc. Natl. Acad. Sci.*, 97: 5357-5362.
34. Lo, A.W.I., C.N. Sprung, B. Fouladi, M. Pedram and L. Sabatier *et al.*, 2002. Chromosome instability as a result of double-strand breaks near telomeres in mouse embryonic stem cells. *Mol. Cell. Biol.*, 22: 4836-4850.
35. Sumner, A.T., 1998. Induction of diplochromosomes in mammalian cells by inhibitors of topoisomerase II. *Chromosoma*, 107: 486-490.
36. Sugimoto-Shirasu, K., N.J. Stacey, J. Corsar, K. Roberts and M.C. McCann, 2002. DNA topoisomerase VI is essential for endoreduplication in *Arabidopsis*. *Curr. Biol.*, 12: 1782-1786.
37. Pastor, N., M.J. Flores, I. Dominguez, S. Mateos and F. Cortes, 2002. High yield of endoreduplication induced by ICRF-193: A topoisomerase II catalytic inhibitor. *Mutat. Res./Genet. Toxicol. Environ. Mutagen.*, 516: 113-120.
38. Cortes, F. and N. Pastor, 2003. Induction of endoreduplication by topoisomerase II catalytic inhibitors. *Mutagenesis*, 18: 105-112.
39. Azeddine, E., M. Cunha and M. Kirsch-Volders, 1998. Spindle poisons can induce polyploidy by mitotic slippage and micronucleate mononucleates in the cytokinesis-block assay. *Mutagenesis*, 13: 193-198.
40. Matsuoka, A., K. Matsuura, H. Sakamoto, M. Hayashi and T. Sofuni, 1999. A proposal for a simple way to distinguish aneugens from clastogens in the *in vitro* micronucleus test. *Mutagenesis*, 14: 385-389.
41. Matsushima, T., M. Hayashi, A. Matsuoka, M. Ishidate Jr. and K.F. Miura *et al.*, 1999. Validation study of the *in vitro* micronucleus test in a Chinese hamster lung cell line (CHL/IU). *Mutagenesis*, 14: 569-580.
42. Kirsch-Volders, M., T. Sofuni, M. Aardema, S. Albertini and D. Eastmond *et al.*, 2003. Report from the *in vitro* micronucleus assay working group. *Mutat. Res./Genet. Toxicol. Environ. Mutagen.*, 540: 153-163.