Research Journal of Biological Sciences 12 (1-6): 13-19, 2017
ISSN: 1815-8846

Evaluation of the Genotoxic Effect of *Salvadora persica* on Mice Bone Marrow Cells

Nasser Moh'd Yousef Alshibly
Department of Biology, Faculty of Sciences and Arts, King Abdulaziz University, Khulais, Saudi Arabia

Abstract: The miswak is a typical toothbrush made from the branches of the *Salvadora persica* (Salvadoraceae). In addition, to consolidation the gums, it inhibits tooth decay, eliminating toothaches and break further increase in decay that has already set in. It creates a scent in the mouth, eliminates bad odor, improves the sense of taste and causes the teeth to radiance and shine. The other portions of the tree have therapeutic values as corrective, deobstruent, liver tonic, diuretic, analgesic, anthelmintic, astringent, lithontriptic, carminative, diuretic, aphrodisiac and stomachic. The present review is therefore an effort to give detailed survey of the literature on phytochemistry and pharmacological activities of miswak. Genotoxicity of *Salvadora persica* was evaluated on mice bone marrow cells using cytogenetics and molecular biology markers. Micronuclei in Polychromatic Erythrocytes (PCEs), Chromosome Aberrations (CAs) in bone-marrow and types of damage in DNA with Comet assay were scored after treatment of mice with 0.325, 0.65 and 1.3 mg/kg BWT for 5 successive days. The results showed significant increase in numbers of micronuclei, types of chromosome aberration in bone-marrow cells in treated groups compared with control groups. In addition, significant differences in levels of damage in DNA of both liver and bone-marrow cells in treated groups compared with control groups. The study reveals that high doses of *Salvadora persica* induce features of genotoxicity in whit mice. The researchers recommend that extreme supervision must be applied upon uncontrolled using of *Salvadora persica* and the high doses must not be used except when highly needed.

Key words: *Salvadora persica*, genotoxicity, natural products, chromosomal aberrations, sister chromatid exchanges, mice

INTRODUCTION

Plants are natural source of antibacterial agents. Plant-derived medicines have been a part of the traditional health care system and the antimicrobial properties of plant derived compounds are well documented. Herbal medicines are more effective and less harmful as they have negligible side effects. They exhibit low mammalian toxicity and can be handled easily (Deshpande *et al*. 2011).

The toothbrush tree, *Salvadora persica* L. locally called miswak is a member of the Salvadoraceae family has been used by many Islamic communities, as toothbrushes and has been scientifically confirmed to be very useful in the prevention of tooth decay, even when used without any other tooth cleaning methods (Salehi and Danaie, 2006). Chewing sticks that are made from the roots, twigs or stems of *S. persica* are commonly used in the Middle East, as a means of maintaining oral hygiene. Studies indicate that *S. persica* extract is somewhat comparable to other oral disinfectants and anti-plaque agents, such as triclosan and chlorhexidine gluconate if used at a very high concentration (Almas, 2002; Almas *et al*., 2005). It has been reported that extracts of miswak posses various biological properties, including significant antibacterial (Al-Lafi and Ababneh, 1995), antifungal (Al-Bagieh *et al*., 1993) and anti-plasmodial effects (Ali *et al*., 2002). *S. persica* and other related plants are reported to be effective against bacteria that are important for the development of dental plaque.

A phytochemical investigation of stems from *S. persica* by Khalil resulted in the 1st isolation of 4 benzylamides from a natural source. The isolated compounds were identified, as butanediamide, N1, N4-bis (phenylmethyl)-2(S)-hydroxy-butanediamide, N-benzyl-2-phenylacetamide, N-benzylbenzamide and benzylurea (Khalil, 2006). Phytochemical investigation revealed that it contains oleic, linolic and stearic acids. Among the compounds identified are esters of fatty acids and of aromatic acids and some terpenoids (Abd Elrahman *et al*.,

Corresponding Author: Nasser Moh'd Yousef Alshibly, Department of Biology, Faculty of Sciences and Arts, King Abdulaziz University, Khulais, Saudi Arabia

2003). The major components from the essential oil of the toothbrush tree *S. persica* stem have been identified as 1,8-cineole (eucalyptol) (46%), a-caryophellene (13.4%), b-pinene (6.3%) and 9-epi-(E)-caryophellene (Alali *et al*., 2005). GC-MS analysis of the volatile oil extracted from *S. persica* leaves revealed benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene and b-caryophyllene as important constituents (Alali and Al-Lafi, 2003). Sticks from *S. persica* have been analyzed for their soluble and total content of fluoride, calcium, phosphorus and silica. There was a substantial amount of silica in the ashes of miswak (Hattab, 1997).

The tooth brush tree, *Salvadora persica* L. locally called miswak is a member of the Salvadoraceae family, has been used by many Islamic communities as toothbrushes and has been scientifically proven to be very useful in the prevention of tooth decay, even when used without any other tooth cleaning methods (Salehi and Danaie, 2006). Chewing sticks that are made from the roots, twigs or stems of *S. persica* are commonly used in the Middle East as a means of maintaining oral hygiene. Studies indicate that *S. persica* extract is comparable to other oral disinfectants and anti-plaque agents, such as triclosan and chlorhexidine gluconate if used at a very high concentration (Almas, 2002; Almas *et al*., 2005).

Genotoxicity is a special area in toxicities and often the most difficult to detect. It may be defined as a chemically induced mutation or alteration of the structure and/or segregation of genetic material (Ifeoma and Oluwakanyinsola, 2013). Many tests exist to evaluate the genotoxic effect of herbs: Gene mutation tests, chromosomal aberration tests and DNA damage tests (Ames *et al*., 1990). Chromosomal aberrations and other forms of DNA damage are the cause of many human genetic diseases. There is substantial evidence that chromosomal damage is involved in cancer development in experimental animals and humans (Chipault *et al*., 1956).

The aim of this study was to evaluate the genotoxic effect of a leaf extract of *Salvadora persica* on the, using a mice model to test for the induction of chromosomal aberrations and its effect on cells mitotic index.

MATERIALS AND METHODS

Plant material collection and preparation: Aerial parts of *Salvadora persica* plants were collected during their growing season in April. 2013. The plant was identified by a taxonomist In order to prevent welting and shriveling, the collected plants were pressed flat on an absorbent surface for several days in a warm, sunny position and where air circulation has been maintained. When completely dried, the leaves were separated from stems carefully and then grounded using an electric blinder (IKA-Werke, Germany) until a fine powder was obtained. The plant material was sealed in labeled dark bottles and then kept in a dark and totally dry cabinet at room temperature (20-25°C) for safe preservation until being used for a period of no >1 week. Each 500 g of this powder was extracted by ethanol-water mixture (70/30 V/V) for 48 h. This step was repeated for 3 times then the filtrate was then refluxed in (2 L) 70% ethanol at 50°C using a rotary evaporator for 36 h in continuous extraction (Soxhlet) apparatus (S.P. Verma-Popular Science Apparatus Workshops Pvt Itd-India). Pooled and concentrated ethanol extract was filtered and it was re-concentrated under reduced vacuum pressure keeping a constant temperature <50°C. The concentrate yield from this process was 80 g which was kept in room temperature for later use.

All *S. persica* extracts were suspended in DMSO at a concentration of 150 mg/mL. The stock solution was kept in a freezer at -200C. Working dilutions were made in physiological saline at a pH of 7.4.

Preparation of doses: A series of descending concentrations (1000, 500, 250 and 125 mg/kg, respectively) were prepared based on the LD50 which was estimated to be 2000 mg/kg. All dosing solutions administered were at a volume of 0.2 mL. The 30 mg was considered safe for injecting mice of (25-30 g) Body Weight (BW) producing a concentration of 1200 mg/kg which was the highest concentration used. The lower doses were prepared by diluting the stock with measured volumes of DMSO. These concentrations were applied in order to investigate the genotoxic effect of extracts on mice bone marrow cells.

Preparation of mice bone marrow cells: Total 96 swiss mice, 10-12 weeks old were randomly divided into 8 groups. Total 4 groups were considered as the controls while the other 4 groups were the ones to be treated with the drug to be tested. Total 12 mice were injected Intraperitoneally (I.P) with 4 different concentrations of *Salvadora persica* (1000, 500, 250 and 125 mg/kg) while the control groups received an equivalent volume of DMSO, for 4 different time intervals (8, 24, 48 and 72 h). A triplicate for each dose level was done for each of the time intervals mentioned previously.

Bone marrow cells were isolated according to (Rossi *et al*., 1987). The mice were humanely sacrificed by cervical dislocation, 1-2 h after injecting them I.P with 0.025 g/kg BW colchicine (Sigma, USA). Femora were removed, cleaned with 70% ethanol and any excess muscle and fat tissues were trimmed carefully without harming the bone's integrity.

Mice bone marrow cells harvesting: Bone marrow cells preparation was made according to the method by Luke and Tice (1989) with some modifications. An opening into the bone marrow cavity was made by cutting the ends of each bone. Next, the bone marrow was flushed using a syringe filled with pre-warmed (37°C) sterile phosphate buffer saline solution (PBS, 0.1 M, pH 7.2), (Bio basic, Canada) into a sterile 15 mL conical tube. The cells were then centrifuged at 10000 rpm for 10 min. The supernatant was discarded and the cell pellet was suspended in 8 mL of warm (37°C) hypotonic solution of potassium chloride (0.075M) (Gainland, UK) and incubated at 37°C for 25 min. Next, the swollen cells were centrifuged for 10 min at 10000 rpm, the supernatant was carefully aspirated without disturbing the cell button. The cells were fixed in 9 mL of cold, freshly prepared carnoy's fixative (absolute methanol: glacial acetic acid 3:1 (v\v)) (Gainland, UK) drop by drop with gentle vortixing and left for 30 min at 4°C, then centrifuged at 10000 rpm for 10 min. The supernatant was discarded and fixation procedure was repeated twice. Cells were resuspended in a volume of 2 mL of the fixative to give an appropriate cell density, then stored at 4°C until being used for slides preparation.

Chromosomes spreading, staining and slide analysis: Chromosome spreading and slide staining were accomplished according to Allen *et al.* (1978). Fixed cells were resuspended and dropped onto wet, precleaned and chilled slides (slides were soaked in 70% methanol and kept at 4°C for 24 h). Slides were passed over Bunsen burner 3-4 times, heated in an oven at 40°C for 20 min, stained using 15% Giemsa stain (Fluka, USA) for 15 min and then rinsed with distilled water.

The slides were analyzed using a light microscope (Motic, Uk). The following parameters were the soul of the investigation.

Chromosomal aberrations: A range of (2000-4000) nuclei was examined for structurally aberrant chromosomes including: Ring chromosomes, chromosomal breaks, sticky chromosomes and polyploidy chromosomes, according to Cunha *et al.* (1997). Chromosomal aberrations were calculated according to the following equation:

$$\text{Chromosomal Aberrations (CA\%)} = \frac{\text{Total no. of chromosomal aberration}}{\text{Total no. of cells examined}}$$

Mitotic index: A range of (2000-4000) cells were scored and the mitotic index was calculated, as the number of cells in division expressed as a percentage of the total number of cells observed, according to the following equation by Al-Azzawi (2012):

$$\text{Mitotic Index (MI)} = \frac{\text{No. of divided cells}}{\text{Total no. of cells}} \times 100$$

Statistical analysis: Statistical analysis was carried out using a statistical analysis program (Graphpad Prism, Version 5 USA). Results were expressed as mean± Standard Deviation (SD) or Standard Error of the Mean (SEM). One-way Analysis of Variance (ANOVA) was used to test for significant differences between mean values and Bonferroni post-tests. A value of $p \leq 0.05$ was considered as statistically significant. Genotoxicty test, researchers used student t-test while for the cytotoxicity test, one-way ANOVA was used.

RESULTS AND DISCUSSION

Chromosomal aberrations: The ability of different concentrations of alkaloids extracted from *Salvadora persica* to induce an adverse cytological effect was assessed, based on the formation of chromosomal aberrations in swiss male mice bone marrow cells. Different concentrations of *Salvadora persica* extract (125, 250, 500 and 1000 mg/kg BW) were interperitonealy injected for 4 different time intervals (8, 24, 48 and 72 h) following the injection. Then, chromosomal aberrations were evaluated by scanning 4000 mice bone marrow cells.

In all treatments, the most frequent chromosomal aberrations observed were sticky chromosomes, followed by polyploidy cells, break and ring chromosomes (Fig. 1). However, cells with >1 chromosomal aberration were not observed.

Statistical analysis of the data revealed that after 8, 24 and 48 h (Fig. 1) of treating the mice with different oncentrations, none of the doses showed a significant difference with respect to the control group (ANOVA, $p>0.05$) even though there has been a time and dose dependent increase in the values. After 72 h of treatment with alkaloidal fractions, a significant increase in chromosomal aberrations was noticed with the concentration of (500 and 1000 mg/kg BW) ($p<0.05$) (Table 1 and 2).

Genetic toxicology is concerned primarily with the mutation effects of chemicals. Mutation refers to a genetic

Table 1: Chromosomal aberrations of mice bone marrow cells induced by *Salvadora persica* extract (125, 250, 500 and 1000 mg/kg) for 8, 24, 48 and 72 h

	Chromosomal aberration (%)			
Dose (mg/kg)	8 h	24 h	48 h	72 h
DMSO	0.74	0.75	1.17	1.44
125	0.76	0.78	1.57	2.24
250	1.06	1.19	1.76	2.50
500	1.40	1.42	2.27	3.27
1000	1.79	1.83	2.59	3.96

($p>0.05$) compare to control; after 72 h; $p \leq 0.05$

Table 2: Effect of different concentrations (125, 250, 500 and 1000 mg/kg) of *Salvadora persica* extract on the cell division of mice bone marrow cells, after 8, 24, 48 and 72h

	Number of dividing cell			
Dose (mg/kg)	8 h	24 h	48 h	72 h
DMSO	48.64	47.86	47.31	47.03
125	44.63	43.19	41.48	37.81
250	40.85	38.51	36.92	34.74
500	37.74	35.58	33.73	23.48
1000	35.23	31.26	29.60	17.29

($p>0.05$) compare to control; after 72 h; $p\leq0.05$

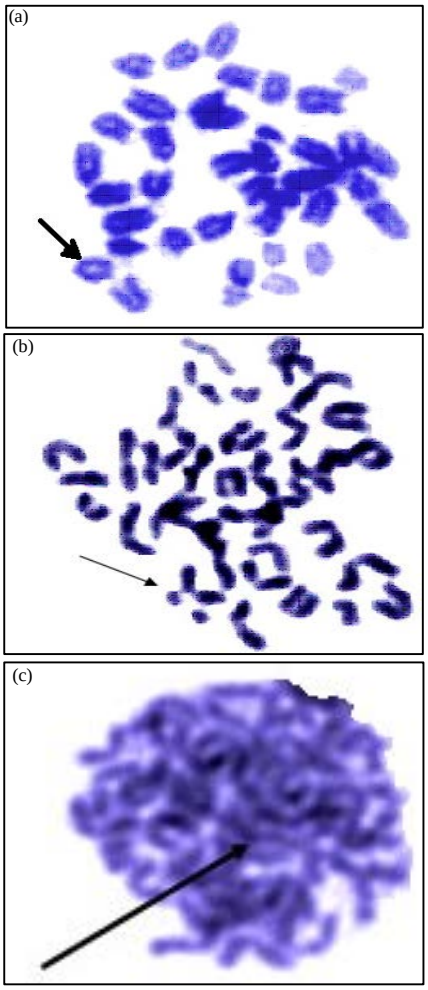

Fig. 1: Types of Chromosomal Aberrations observed after treating mice with *Salvadora persica* extract. The bar measures 5 μm in diameter; a) Ring chromosomes; b) Sticky chromosomes and c) Chromosomes break

alteration in somatic (body) or germ cells. The mutations in somatic cells may contribute to various defects including cancer while the mutations in germ cells cause potential genetic disease in future generations (Wassom, 1989; Kim and Margolin, 1999).

Cancer is considered one of the major human diseases, causing considerable suffering and economic loss all over the globe. Cancer can occur in all living cells in the body that may be induced by inherited and acquired susceptibility factors on exposure to initiation factors (exogenous and endogenous carcinogens) and on promotion and progression factors (Witschi *et al*., 2004). One of the major limitations is cancer chemoprevention which targets normal and high risk populations. It involves the use of drugs or other chemical agents to inhibit, delay or reverse cancer development (Kelloff *et al*., 1999). At present, the focus of chemoprevention of cancer research includes the identification, characterization and development of a new and safe cancer chemopreventive agent (Kelloff, 2000).

Chromosomal aberration assays are considered to be very sensitive end points recognizing the genotoxic induced by chemicals (Sweify *et al*., 2005). With the objective of investigating the possible induction of chromosomal aberrations by different concentrations of *Salvadora persic*a extract for various periods of time (8, 24, 48 and 72 h) a cytological analysis was conducted using albino swiss mice bone marrow cells. Several cytological parameters were observed during the analysis while the most prominent were sticky chromosomes; polyploidy, ring and chromosomal breaks were less frequent.

Salvadora persica and its constituents have been extensively studied against cancer during the last decade. Steiner *et al*. (2001) found that 10 μM of carsonic acid prevented cell proliferation in HL60 cells and caused transient G_0/G_1 phase cell cycle arrest. In another study, the effect of carsonic acid and carnosol on growth of Caco-2 (colonic adenocarcinoma cell lines) was determined and found that (H^3) thymidine incorporation is inhibited in a concentration-dependent way (Steiner *et al*., 2001). This study demonstrated that carnosic acid reduced the level of cyclin A that would decrease the activity of CDK2 (Cyclin-Dependent Kinase 2) and CDC2 (Cell Division Cycle 2) kinases, thus inducing cell cycle arrest at G_2/M phase before prometaphase or late S phase and preventing the cells from exiting mitosis.

Amongst the changes which affect chromosome structure in abnormal cells, the most common is stickiness of the chromosomes which can be easily noticed during anaphase. This type of abnormality happens when chromosomes stick together at the end and lag behind the normally segregating chromosomes. Sticky chromosomes form bridges stretching between the poles of the cell, thus, preventing the separation of the telophase chromosome groups. Cell division very often remains

incomplete and a loss of chromosome material in these cells occurs very frequently (Koller, 1947). As mentioned previously this type of CA was observed most abundantly during the analysis which comes in agreement with the findings of Tawab who reported that spindle disturbance was the most common abnormality that occurred as a result of treatment with *R. officinalis* water extract at various levels (Tawab *et al*., 2004).

Other types of chromosomal aberrations were observed, as well but in low frequencies including chromosomal breaks which result from the action on the DNA synthesis. It is a dangerous kind of DNA lesion that can interrupt the coding sequence of a gene, disrupt the linkage between coding and regulatory sequences, alter chromosome organization and perturb the systems that ensure correct DNA replication, chromosome packaging and chromosome segregation (Cromie *et al*., 2001). 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). Ring chromosomes are also types of CA which results from stickiness and double strand breakage (lesions) and due to an exchange type of interaction which takes place between the 2 lesions after formation of a looped structure (Bryant, 1998).

Polyploidy is the phenomenon of an increase in the chromosome number which usually arises because of the failure of spindle formation. Complete suppression of spindle is common in cells where chromosome synthesis is extremely rapid. Comparison of the frequency of cells in various stages of mitosis in normal and tumor tissues has shown that chromosome synthesis proceeds at a higher rate in tumor cells than in normal cells (Cromie *et al*., 2001).

Decreasing of mitotic index can be explained by the arrest of the division of the interphasic nucleus as well as by death of interfacesnucleus, hindering the onset of prophase and thus the division of the cells (Cooper, 2000). Strachan and Read reported that disrupting the mitotic cycle occurs in 3 ways:

- By inhibiting the process of cell division
- By distrubting the normal functioning of the mitotic spindle
- By producing chromosomal abnormalities (Strachan and Read, 1999)

Thus, the decrease in mitotic index in higher concentrations might be due to the action of alkaloidal compounds on the onset of mitosis which differ from the action of cholchicine in its action. This led us to conclude that alkaloids caused a partial effect on spindle formation. In this respect, it is similar to Kabarity and Malallah (1980).

Furthermore, it has been shown that *Salvadora persica* is a strong mitotic inhibitor and could give rise to mitotic abnormalities with increase in concentration. Their accumulation in cells may be inhibitory to cell growth it has further revealed that *Salvadora persica* can likely be used in mutagenic studies due to their tendency to interfere with DNA biosynthesis.

One major conclusion of the present study is that *Salvadora persica* are genotoxic and may cause cell death if used at high concentrations. Induced the formation of a variety of chromosomal aberrations (ring chromosomes, sticky chromosomes and broken chromosomes) at high percentages in all treatments. These results are of practical interest because *Salvadora persica* is used as a medicinal plant. Therefore, it is important to limit the use of this plant in folk medicine.

These results indicate that it is important to do further studies to identify the active alkaloidal compound(s) responsible in its mutagenicity and to test the effect of higher concentrations on mitotic index and cell growth as well as using other cytogenetic methods such as flouorescence in situ hybridization, comet assay and the micronucleus assay.

CONCLUSION

It is concluded that miswak (*S. persica*) reduces the microbial count in different groups and improves the oral health. The extract possesses antibacterial and antiplaque property and it can be used effectively as a natural tool for teeth cleansing and as a natural analgesic for the disturbing toothache. The drug is also reported to possess anti-inflammatory, anticonvulsant, sedative, antiulcer, hypolipidemic and hypoglycemic activities. The present review showed that it is useful in a number of diseases. Therefore, it is imperative that more clinical and pharmacological studies should be conducted to investigate unexploited potential of this plant. The research workers have isolated many phytoconstituents from the plant. Nevertheless, further investigations are required to isolate and purify novel pharmacologically active and industrially important compounds.

REFERENCES

Abd Elrahman, H.F., N. Skaug, A.M. Whyatt and G.W. Francis, 2003. Volatile compounds in crude *Salvadora persica* extracts. Pharm. Biol., 41: 399-404.

Al-Azzawi, A.M., 2012. Genotoxic and cytotoxic study of *Tecoma stans* bignoniaceae. Pak. J. Biol. Sci., 15: 92-97.

Al-Bagieh, N.H., A. Idowu and N.O. Salako, 1993. Effect of aqueous extract of miswak on the *in vitro* growth of *Candida albicans*. Microbios, 80: 107-113.

Al-Lafi, T. and H. Ababneh, 1995. The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. Int. Dent. J., 45: 218-222.

Alali, F. and T. Al-Lafi, 2003. GC-MS analysis and bioactivity testing of the volatile oil from the leaves of the toothbrush tree *Salvadora persica* L. Nat. Prod. Res., 17: 189-194.

Alali, F., M. Hudaib, T. Aburjai, K. Khairallah and N. Al-Hadidi, 2005. GC-MS analysis and antimicrobial activity of the essential oil from the stem of the Jordanian toothbrush tree *Salvadora persica*. Pharmaceut. Biol., 42: 577-580.

Ali, H., G.M. Konig, S.A. Khalid, A.D. Wright and R. Kaminsky, 2002. Evaluation of selected Sudanese medicinal plants for their *in vitro* activity against hemoflagellates, selected bacteria, HIV-1-RT and tyrosine kinase inhibitory and for cytotoxicity. J. Ethnopharmacol., 83: 219-228.

Allen, J., C. Shuler and S. Latt, 1978. Bromodeoxyuridine tablet methodology for *in vivo* study of DNA synthesis. Somatic Cell Genet., 4: 393-405.

Almas, K., 2002. The effect of *Salvadora persica* extract (miswak) and chlorhexidine gluconate on human dentin: A SEM study. J. Contemp. Dental Pract., 3: 27-35.

Almas, K., N. Skaug and I. Ahmad, 2005. An *in vitro* antimicrobial comparison of miswak extract with commercially available non-alcohol mouthrinses. Int. J. Dent. Hyg., 3: 18-24.

Ames, B.N., M. Profet and L.S. Gold, 1990. Nature's chemicals and synthetic chemicals: Comparative toxicology. Proc. Natl. Acad. Sci., 87: 7782-7786.

Bryant, P.E., 1998. Mechanisms of radiation-induced chromatid breaks. Mutat. Res. Fundam. Mol. Mech. Mutagen., 404: 107-111.

Chipault, J.R., G.R. Mizuno and W.O. Lundberg, 1956. The antioxidant properties of spices in foods. Food Technol., 10: 209-211.

Cooper, G.M., 2000. The Cell: A Molecular Approach. 2nd Edn., Sinauer Associates Inc., Sunderland, MA., USA.

Cromie, G.A., J.C. Connelly and D.R. Leach, 2001. Recombination at double-strand breaks and DNA ends: Conserved mechanisms from phage to humans. Mol. Cell, 8: 1163-1174.

Cunha, R.A.F., C.P. Koiffman, D.H. Souza and K. Takei, 1997. Clastogentic effects of different Ureaplasma urealyticum serovars on human chromosomes. Braz. J. Med. Biol. Res., 30: 749-757.

Deshpande, R.R., A.A. Kale, A.D. Ruikar, P.S. Panvalkar, A.A. Kulkarni, N.R. Deshpande and J.P. Salvekar, 2011. Antimicrobial activity of different extracts of *Juglans regia* L. against oral microflora. Int. J. Pharm. Pharm. Sci., 3: 200-201.

Hattab, F.N., 1997. Meswak: The natural toothbrush. J. Clin. Dent., 8: 125-129.

Ifeoma, O. and S. Oluwakanyinsola, 2013. Screening of Herbal Medicines for Potential Toxicities. In: New Insights into Toxicity and Drug Testing, Gowder, S. (Ed.). InTech, USA., ISBN-13: 9789535109464.

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

Kelloff, G.J., 2000. Perspectives on cancer chemoprevention research and drug development. Adv. Cancer Res., 78: 199-334.

Kelloff, G.J., C.C. Sigman and P. Greenwald, 1999. Cancer chemoprevention: Progress and promise. Eur. J. Cancer, 35: 1755-1762.

Khalil, A.T., 2006. Benzylamides from *Salvadora persica*. Arch. Pharm. Res., 29: 952-956.

Kim, B.S. and B.H. Margolin, 1999. Prediction of rodent carcinogenicity utilizing a battery of *in vitro* and *in vivo* genotoxicity tests. Environ. Mol. Mutagen., 34: 297-304.

Koller, P.C., 1947. Abnormal mitosis in tumours. Br. J. Cancer, 1: 38-47.

Luke, C.A. and R.R. Tice, 1989. Effect of processing time on the quality of mouse bone-marrow metaphase preparations. Mutat. Res. Lett., 227: 59-62.

Palitti, F., 1998. Mechanisms of the origin of chromosomal aberrations. Mutat. Res., 404: 133-137.

Rossi, A.M., M. Romano, L. Zaccaro, R. Pulci and M. Salmona, 1987. DNA synthesis, mitotic index, drug-metabolising systems and cytogenetic analysis in regenerating rat liver: Comparison with bone marrow test after *in vivo* treatment with cyclophosphamide. Mutat. Res., 182: 75-82.

Salehi, P. and S.M. Danaie, 2006. Comparison of the antibacterial effects of persica mouthwash with chlorhexidine on streptococcus mutans in orthodontic patients. Daru, 14: 178-182.

Steiner, M., I. Priel, J. Giat, J. Levy, Y. Sharoni and M. Danilenko, 2001. Carnosic acid inhibits proliferation and augments differentiation of human leukemic cells induced by 1, 25-dihydroxyvitamin dsub3 and retinoic acid Nutr. Cancer, 41: 135-144.

Strachan, T. and A.P. Read, 1999. Human Molecular Genetics. 2nd Edn., Wiley, New York.

Sweify, K.M., I.A. Darwish and S.A. Mosallam, 2005. The cytogenetic effect of the new asthma and allergy drug montelukast on albino mice: Chromosomal studies. Egypt. J. Biol., 7: 33-43.

Tawab, S.A., Z.M. Adam and S.H. Selmi, 2004. Suppression of mitotic process associated with metaphase arrest of *Allium cepa* L. roots using: I. *Rosmarinus officinalis* L. water extract. Int. J. Agric. Biol., 6: 1560-8530.

Wassom, J.S., 1989. Origins of genetic toxicology and the environmental mutagen society. Environ. Mol. Mutagen., 14: 1-6.

Witschi, H., I. Espiritu, D. Uyeminami, K.E. Pinkerton and O.G. Raabe, 2004. Chemoprevention of tobacco smoke-induced lung cancer: What animal studies tell us. Chest, 125: S128-S128.