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Assessment of the Genotoxicity, Antigenotoxicity on Alloxan-Induce Diabetic in Mice by Micronuclus(MN) Assay

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Abstract
Fenugreek (*Trigonellafoenum-graecum*) plant has become interest subject because of its beneficial effects on human health, good nutritive value in addition it has antigenotoxic effects therefore, the purpose of the present study was to investigate antigenotoxic effects of alcoholic extract of Fenugreek seed at 200 mg /kg b.w on mice bone marrow which treatmed with alloxan, 6-mercaptopurine anticancer at 200 mg /kg b.w by using micronuclus test at 5 day for all treatments. Cytogenetic study showed significant increasing at ($p< 0.05$) in Micronuclus number MN in diabetic mice, anticancergroup or both when compared to the negative control. After 5day of alloxan treated mice with marked hyperglycemia (blood glucose),results recorded significant increase at ($p< 0.05$) in diabetic group (group treated with alloxan) than anticancer in same time compared with control. Finally, alcoholic extracts of Fenugreek seeds found significant reduced MN, blood glucose in all treated groups. These results indicate that fenugreek plant were one of the primary supplements used to support type I diabetics or insulin-dependent diabetes mellitus (NIDDM), in addition it has active compounds which have important role on antigenotoxic, hyperglycemia activity.
Keywords: Extracts,Fenugreek,Alloxan,Micronuclus assay ,anticancer,mercaptopurine.

Introduction

Micronuclei (MN) and other nuclear anomalies such asnucleoplasmic bridges (NPBs) and nuclear buds (NBUDs)are biomarkers of genotoxic events and chromosomal instability **[1].** Micronucleus refers to the fragment of damaged chromosomes or whole chromosomes which fail to find their way onto the spindle during cell division, which are much smaller than the principle nuclei and are generally referred to as micronucleus**[2].** MN represent structural chromosomal maberrations (chromosome loss or breakage) induced by ionizing radiation or chemical mutagens **[3].**

Genotoxicity of anticancer drugs to normal cells is one of the most serious problems of chemotherapy due to the possibility of inducing secondary malignancies **[4]** and it is no doubt that DNA damage plays an important role in most mechanisms underlying the action of anticancer drugs interacting with DNA. It is therefore an imperative task in chemotherapy to determine the DNA-damaging effect of these drugs on normal cells **[5, 6, 7].** A number of medicines drugs used anticancer treatment cause Cytotoxicity and Genotoxicity, but most of the anticancer drugs target the enzyme systems in the cell cycle to block cell division **[8, 9].** Six-mercaptopurine (6-MP) has been used in cancer chemotherapy, primarily in childhood and adult leukemia and usually in combination with other drugs. 6-MP is also used to treat autoimmune diseases, such as inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) **[10,11].** Azathioprine (AZA) and its active metabolite 6-mercaptopurine(6-MP) drugs are not only cytotoxic but also immunosuppressive and anti inflammatory. The effects are dose-related, small doses of either drug are anti-inflammatory, but larger doses are immunosuppressive and cytotoxic **[12].** In humans, *diabetes mellitus* is one of the most prevalent conditions with spontaneous manifestation. In animals, it can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan, streptozotocin, ditizona and anti-insulin serum **[13]** . These agents selectively destroy the Langerhans islet ß-cells. Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil), a derivative of uric acid, as well as of other substances of different chemical groups causes ß-cells to degranulate and consequently degenerate **[14,15,16].** The dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Human islets are considerably more resistant to alloxan thanthose of the rat and mouse **[17].**DNA damage and repair play a major role in neoplastic transformation, because mutations in DNA repair genes can be directly related with cancer and the efficacy of DNA repair may determine the susceptibility to carcinogenesis **[18].**Treatment of diabetes mellitus and its complications in the recent context has focused on the usage of plant extracts and their constituents **[19].**

In many industrialized countries herbal medicines are gaining popularity as alternative and complimentary therapies **[20].**Some of the plants are exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-diabetic, and anti-microbial functions**[21].**

Fenugreek (*Trigonellafoenum-graecum*) is a member of the Leguminosae (Fabaceae) family and is commonly cultivated in India, Egypt, the Middle East and North Africa. The seeds of the plant have been used as a traditional remedy for numerous conditions including gastrointestinal disorders, gout, wound healing and

inflammation, hyperlipidemia and diabetes **[22].**The antihyperglycemic effects of fenugreek seeds and its subfractions are demonstrated in diabetic rats, and also show beneficial effects in cancer**[23,24].** Fenugreek has also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant**[25,26].** Antioxidants are intimately involved in the prevention of cellular damage, the common pathway for cancer, aging, and a variety of diseases by interact with free radicals and terminate the chain reaction before vital molecules are damaged **[27,28].**

The aim of this study is to evaluate the genotoxic activity of mice using micronucleus (MN) test on DNA damage induced by 6-mercaptopurine anticancer drugs and alloxan induced-diabetic mouse bone marrow cells *in vivo* and evaluate anti-genotoxic activity by alcohol extracts of Fenugreek seeds**.**

Materials and Methods

Animals: Males of albino mice weighing 12-20 gram were maintained under standard laboratory conditions and provided a standard diet and water *ad libitum*.

Chemicals: Anti-cancer: The anti-cancer drugs 6-mercaptopurine (Purinethol) Was selected for present study due to their is mutagenic in animals and humans.

Alloxan (diabetes Induction) was obtained from CDH-Central Drug House – India.

Plant Extracts Preparation: Preparation of alcoholic extract of Fenugreek seed according Jin *et al.,*[**29].**The seeds air dried in the shade, grounded into a fine powder by using coffee grinder and weighing (100 gm) then put it in a volumetric conical flask then 1000 ml of 70 % ethyl alcohol was added on the powder which make the ratio (1/10) (W/V). After that the mixture was shake by using magnetic stirrer apparatus for 24 hours, the mixture was filtered by using 4 layers of medical gauze then was filtered again using what man NO.1 filter paper. The filtrated mixture was concentrated by using incubator on (40 C°) for 72 hr, to obtain crude extract. This extract was stored in dark sterile screw bottle in (40C°) until use.

Experimental design:

This experiment was carried out to assess the genotoxic analysis of Mercaptopurine ,Alloxan – induce diabetes and Anti-genotoxic effect of alcoholc extract of Fenugreek. The male mice were divided in to six experimental groups. Each group consisted of 4 male mice. The animal were divided to:

Group **I**: As negative control treated with 0.5 ml of distill water injected intra preitoneal for 5 day .

Group **II**: As positive control treated with 200 mg /kg b.w of Alloxan injected intra pritoneal for 5 day.

Group **III**: As positive control treated with 200 mg /kg b.w of Mercaptopurine injected intrapreitoneal for 5 day.

Group **IV**: Each animal had treated with 200 mg /kg b.w of Mercaptopurine injected intra peritonial then treated with Alloxan by injected intra peritoneal 200 mg /kg b.w for 5 day.

Group**V**: Each animal had treated with 200 mg /kg b.w of Mercaptopurine injected intra peritoneal then intra peritoneal injection 200 mg /kg b.w of Fenugreek intra preitoneal for 5 day.

Group **VI**: Each animal had treated with 200 mg /kg B.W of Mercaptopurine intra peritonial, intra peritoneal injection 200 mg /kg B.W of Alloxanand and 200 mg /kg b.w of Fenugreek extract intra preitoneal for 5 day.

Cytogenetic Experiments-Micronucleus test in mouse bone marrow cells

The experiment was done according to method of **[30]** as follow :

1-The femur bone cleaned from tissues and muscles, then gapped from the middle with a forceps in a vertical position over the edge of a test tube by a sterile syringe, (1 ml) of bovine calf serum (heat inactivated) was injected so as to wash and drop the bone marrow in the test tube.

2-The test tubes were centrifuged at speed of 1000 rpm (5 min).

3-The supernatant was removed, and one drop from the pellet was taken to make a smear on a clean slide. The slides were kept at room temperature for (24 hr).

4-The slides were fixed with absolute methanol for (5 min.), then stained with Giemsa stain for (15 min), then washed with D.W and left to dry.

5- Two slides for each animal were prepared for micronucleus test.

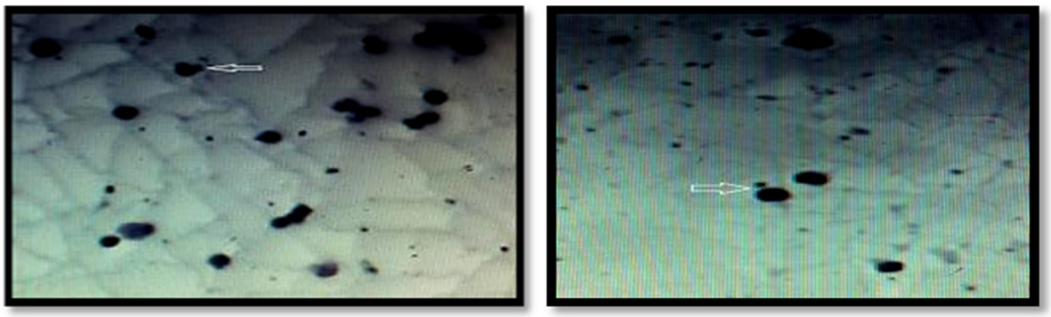
Statistical analysis

Statistical analysis was performed with SPSS software. Data were analyzed using three-way analysis of paired-samples T-test for comparison between different treatment. Results were reported as mean ± S.E. and differences were considered as significant when $P<0.05$.

Results

In order to evaluate the protective, antigenotoxicity or therapeutic effects of Fenugreek (*Trigonellafoenum-graecum*) , mice were pretreated for 5day with alloxan, anticancer or both .Results in table (1) showed significant increase MN on mice bone marrow at ($p< 0.05$) of group treated with alloxan , anticancer or both(4.56 ± 0.010 , 4.18 ± 0.058**,** 4.20 ± 0.044) frequency compared to the control (1.16 ± 0.024**)** . Symptoms which were observed

in the animals during treatment with alloxan and anticancer or both: decreased activity, closed eyes, diarrhoea and tremors.Results showed significant increase MN on mice bone marrow in all groups treated with alloxan , anticancer or both (figure 1).



Figure(1): Micronucleus cell at 40X magnification

After 5 day of alloxan treated , mice with marked hyperglycemia, table (2) recorded significant increase at ($p< 0.05$) in diabetic group 378.80 ± 22.352 compared with control (153.80 ± 5.053).

In this works showed significant different for all groups when treated with Fenugreek alcoholic extracts, table (1) showed that fenugreek extracts caused significant reduce MN when treated with anticancer group (1.50 ± 0.148) compared with only anticancer group (4.18 ± 0.058 **).**Fenugreek plant have hyperglycemia activity, table (2) showed Fenugreek alcoholic extracts significant reduced blood glucose in group treated with alloxan, anticancer in same time (145.80 ± 3.023) compared with alloxan and anticancer treated group (472.00 ± 23.323 **).**

Table (1): Micronuclus assay MN of experimental mice (Mean ± S.E.).

Group	Treatment	MN / 1000 Cell (Mean ± S.E.)
I	Control	1.16 ± 0.024 [a]
II	Alloxan	4.56 ± 0.010 [a]
III	Anticancer	4.18 ± 0.058[a b]
IV	Anticancer Alloxan+	4.20 ± 0.044 [b]
V	Anticancer + Extract	1.50 ± 0.148 [a b c]
VI	Anticancer +Extract+Alloxan	2.10 ± 0.031 [a b d]

Means with different superscript letters are significantly different (P<0.05).

Table (2): blood glucose level of experimental mice (Mean ± S.E.).

Group	Treatment	Blood Glucose level (Mean ± S.E.)
I	Control	153.80 ± 5.053 [a]
II	Alloxan	378.80 ± 22.352 [a b]
III	Anticancer	151.40 ± 3.140 [a c b]
IV	Anticancer +Alloxan	472.00 ± 23.323 [a b c]
V	Anticancer + Extract	144.00 ± 4.301[a b c]
VI	Anticancer +Alloxan +Extract	145.80 ± 3.023 [b c]

Means with different superscript letters are significantly different (P<0.05).

Discussion

Table (1) showed significant increase MN number on mice bone marrow at ($p< 0.05$) of group treated with alloxan ,

anticancer or both when compared compared with control .

Joubori *et al*.,[**31]** who mention that alloxan may cause increasing in insulin like growth factor- 1(IGF-1) or insulin like growth factor-2(IGF-2). Insulin receptor (IR) of bone marrow cell may respond to another factor (other than insulin due to deficiency of insulin in diabetic rats) as insulin –like growth factor-2 (IGF-2) which bind with IR-A to send mitogenic, anti apoptotic signals. micronucleus formations, Blasiak *et al* [**32]** recorded that spermatocyte chromosomal aberrations, and sperm characteristic assays to investigate the chromosomal instability in somatic and germinal cells of diabetic rats treated.

Some reports suggest the diabetes can be associated with cancer, but the mechanism underlying this association is unclear. A previous study demonstrated that diabetes was linked with the elevated level of oxidative DNA damage, which increased susceptibility to mutagens, and the decreased efficacy of DNA repair **[33].** Because oxidative DNA damage may contribute to cancer promotion and progression, it can be considered as an element of the link between diabetes and cancer **[34].**

Vijayalaxmi and Souza **[35]** reported increased chromosomal aberration and MN number in the bone marrow cells of the mice treated with the Taxol anticancer drug in triple therapeutic dose. Carboplatin antitumor drug induced significant increased MN both in the fetal liver and the maternal bone marrow at different doses**[36].**

It is known that the micronuclei originates either from fragment or lagging chromosomes during the cell fission **[37].** This significant increase in MN was not accompanied by significant increase in breaks orfragments. Therefore, the formation of MN was not attributed to chromosome fragments but to lagging chromosomes **[38].** Interaction between anticancer drugs (Taxol) and centromere could explain a considerable amount of the centromere positive micronuclei due to multipolar mitosis. As MNs are formed out of whole chromosome and anticancer drugs found to increase significantly the micro-nucleated lymphocyte rates and over 85% of those micronuclei contained one or more whole chromosomes, anticancer drugs is said to be an eugenic **[39].**using natural anti-oxidative compounds would have a benefit in preventing diabetes oxidative stress related consequences, certain antioxidants are known to have genotoxic or carcinogenic potentials **[40].** Our study found that fenugreek alcoholic extracts not have any toxic or lethal side effects on bone marrow cells. That may be due to fenugreek alcoholic extracts have compounds which have hypoglycemic and anti-genotoxicity properties. previous studies showed that fenugreek seeds was not had acute toxicity **[41],[30]** who showed cytogenetic study showed significant increasing in mitotic index and chromosome aberrations in diabetic group while treatment with mixture of plants extracts significantly reduced mitotic index and chromosome aberration in all treatment groups and for different period of study. In the present study, the statistically significant increase of MN number per thousand of in anticancer group was agree with earlier reports **[42, 43].** Our result showed the ability of fenugreek plants extracts to reduced MN number in bone marrow.

Conclusion

fenugreek seeds are one of the primary supplements used to support type I diabetics or insulin-dependent diabetes mellitus (NIDDM). Fenugreek Seed helps to reducing blood sugar levels, and genotoxicity .

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References

1-Fenech, M.; Kirsch-Volders, A. ; Natarajan, J. ;Surralles, J. Crott, J. Parry, Y. ;Norppa, D.;Eastmond, J. and Tucker P. (2011). Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis,26 (1):125–132.

2-Gangar, S.; Sandhir, R. and Koul, A. (2010). Anti-clastogenic activity of azadirachtaindica against benzo(a)pyrene in murine forestomachtumorigenesis bioassay. ActaPoloniaePharmaceutica – Drug Research, 67: 381-390.

3-Fenech, M. (2000). The in vitro micronucleus technique.Mutat Res., 455:81-95.

4-Blasiak, J.; Gloc,E. and Warszawski ,M.(2002). A comparison of the in vitro genotoxicity of anticancer drugs idarubicin and mitoxantrone. ActaBiochimica Polonica,49: 145- 155.

5-Gentile, J.; Ferguson, L.; Rahimi, S. and Gentile, G. (1998). Effect of selected antimutagens on the toxicity of antitumor agents. Mutat Res., 402: 289-298.

6- Li, X. and Chen ,B. (2009). Histone deacetylase inhibitor M344 inhibits cells proliferation and induces apoptosis in human THP-1 Lukemia Cells. Am J Biomed Sci.,1(4): 354-363.

7-Li Xu, Z.; Zhao, Z.; liu, Q.; Tan, W. and Fang, X. (2013). Cellular internalization and cytotoxicity of aptamers selected from lung cancer cells. Am J Biomed Sci., 5(1): 47-58.

8-Suman, G. and Jamil, K. (2006).Application of human lymphocytes for evaluating toxicity of anticancer drugs. Int Journal Pharmacol., 2 (4): 374-381.

9-Kaur, S.; Kumar, S.; Kaurand, P. and Chandel, M. (2010). Study of antimutagenic potential of phytoconstituents isolated from*Termaneliaarjuna in the salmonella/microsome*assay. Am J Biomed Sci., 2(2): 164-177.
10- Bermas, B. and Hill, J. (1995). Effects of immunosuppressive drugs during pregnancy. Arthritis Rheum., 38:1722–1732.
11- Ramsey-Goldman, R. and Schilling, E.(1997). Immunosuppressive drug use during pregnancy. Rheum Dis Clin North Am., 23:149–167.
12-Goldstein, F.(1987). Immunosuppressant therapy of inflammatorybowel disease. Pharmacologic and clinical aspects. J ClinGastroentero,l 9:654–658.
13- Cisternas, J. (2000). Fisiologia das ilhotas de Langerhans. In: Douglas CR. Tratado de fisiologiaaplicada a ciências da saúde. 4th ed. São Paulo:Robe., p. 1073-86.
14- Macedo, C.; Capelletti, S.; Mercadante, M.; Padovani ,C. and Spadella, C. (2002). Role of metabolic control on diabetic nephorathy. Acta Cir Bras.,17 (6): 370-5.
15- Ahrén, R. and Sundkvist, G. (1995). Long Term Effects of Aloxan in Mice. Int J Pancreatol., 2:197-201.
16- Bhattacharya, S. (1995). Activity of shilajit on aloxan induced hypoglicaemia in rats. Fitoterapia.,116(4):328-32.
17-Eizirik, D.; Pipeleers, D. ; Ling, Z.; Welsh, N.; Helerstrom, C. and Andersson, A. (1994). Major species differences between humans and rodents in the susceptibility to pancreatic beta-cell injury. ProcNatlAcadSci USA., 91: 9253-9256.
18- Cousineau, I.; Abaji, C. and Belmaaza,A.(2005).“BRCA1 regulatesRAD51 function in response to DNA damage and suppressesspontaneous sister chromatid replication slippage: implicationsfor sister chromatid cohesion, genome stability, andcarcinogenesis,” Cancer Research., 65(24):11384–11391.
19- Craig, W. 1999. “Health-promoting properties of common herbs,”American Journal of Clinical Nutrition, 70(3): 491.
20- Omobuwajo, O.; Alade, G. and Moody, J. (2011). Microscopic Characters and Phytochemical screening of *Pandanus candelabrum* (P. Beauv., Pandanaceae) Leaves. J. Chem. Pharm. Res., *3(2):98-104.*
21-Donatus, E. and Nnamdi, F.(2011).Two novel flavonoids from *Bryophyllumpinnatum*and their antimicrobial Activity. *J. Chem. Pharm. Res., 3(2):1-10*
22-Fetrow, C. and Avila, J. (1999).in: Professional's Handbook of Complementary & Alternative Medicines.Springhouse Corp, Springhouse, Pa;:372–375.Gabel, “Herbal medications, nutraceuticals, and anxiety and depression,” 211–212.
23- Khosla, P.; Gupta, D.; Nagpal, K.(1995). Effect of Trigonellafoenumgraecum (Fenugreek) on blood glucose in normal and diabetic rats. Indian J PhysiolPharmacol, 39:73–74
24- Sur, P. ; Das, M.; Gomes, A.; Vedasiromoni ,J.; Sahu, N.; Banerjee, S.; Sharma, S.and Ganguly, D. (2001). Trigonellafoenumgraecum (fenugreek) seed extract as an antineoplastic agent .Phytother Res., 15(3):257–259.
25-Marles R. ; Farnsworth, N. (1995). Antidiabetic plants and their active constituents. Phytomedicine. 2 (2) :137-189 .
26-Witt, E.;Z.Reznick,A.;Viguie,C.;Starke-Reed,P.and Packer, L.(1992).Exercise, Oxidative Damage and Effects of Antioxidant Manipulation .Journal of Nutrition 122(3): 766-73.
27-Goldfarb, A. (1993).Antioxidants: role of supplementation to prevent exercise-induced oxidative stress. Medicine and Science in Sports and Exercise. 25(2):232-236.
28- Jin, J.; Koroleva , O. ; Gibson,T.; Swanston, J.; Magan, J. ; Zhang, Y.; Rowland ,I. and Wagstaff,C. (2009).Analysis of phytochemical composition and chemoprotective capacity of rocket (*Eruca sativa* and *Diplotaxistenuifolia*) leafy salad following cultivation in different environments. J. Agric.Food Chem.,57(12): 5227-5234.
29-Schmid, W. (1975) . The micronucleus test. Mutat Res., 31: 9–15.
30-Al-Joubori, M. ; Zaidan, H. and Al Saadi ,A. (2014).Evaluation of Chromosome Aberrations and Mitotic Index in Alloxan-Induced Diabetic Male Rats Treated with the Mixture of Plants Extracts Mixture. Journal of Babylon University .Pure and Applied Sciences. No.(5)/ Vol.(22.
31-Bakheet, S. and Attial, S.(2011). Evaluation of Chromosomal Instability in Diabetic Rats. Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity, Article ID 365292, 9 pages.
32-Blasiak, J. ; Arabski, M.; Krupa ,R.; Wozniak ,K.; Zadrozny, M. and Kasznicki, J. (2004). “DNA damage andrepair in type 2 diabetesmellitus,” Mutation Research., 554(1-2): 297–304.
33-Evans, M. and Cooke, M.(2004). “Factors contributing to theoutcome of oxidative damage to nucleic acids,” BioEssays., 26(5): 533–542.
34-AL-Sharif, M. (2012).Studies on the Genotoxic Effects of Anticancer Drug Paclitaxel (Taxol) in Mice. World Applied Sciences Journal, 16 (7): 989-997.
35-Vijayalaxmi, K. and Souza,M.(2004). Studies on the Genotoxic Effects of Anticancer Drug Carboplatin in in vivo Mouse. Kamla-Raj Int J Hum Genet, 4(4): 249-255 .
36-Alder, I. (1984). Cytogenetic test in mammals. In Mutagenicty testing, a practical a pproache, S. Venilt, and

J.M. Farry, (Eds.) LRL Pres., Oxford.Washington D C.
37-Digue, L.; Orsiere, T.; Bacluchka, M. (2002). Interest of studying the *in vitro* genotoxicity of an antineoplastic drug on healthy human: paclitaxel example Bull. Cancer, 89: 887-892.
38-Goodsell, D. (2000). The molecular perspective: microtubules and the taxanes, Stem Cells, 18: 382-383.
39-Ames, B. (1983). "Dietary carcinogens and anticarcinogens, oxygenradicals and degenerative diseases," Science, 221(4617): 1256–1264.
40-Mowla, A.; Alauddin, M.; Rahman, M.and Ahmed, K. (2009). Antihyperglycemic effect of Trigonellafoenum-graecum (fenugreek) seed extract in alloxan-induced diabetic rats and its use in diabetes mellitus: a brief qualitative phytochemical and acute toxicity test on the extract. Afr J Tradit Complement Altern Med., 6(3):255-261.
41-Kopjar, N.; Garaj-Vrhovac, V. and Milas ,I. (2002). Acute cytogenetic effects of antineoplastic drugs on peripheral blood lymphocytes in cancer patients chromosome aberrations and micronuclei, Tumori., 88: 300-312.
42- Gupta, N.; Hu, L. and Deen, D. (1997).Cytotoxicity and cell-cycle effects of paclitaxel when used as a single agent and in combination with ionizing radiation. Int. J. RadiatOncol. Biol. Phys., 37(4): 885-895.
43-Gorodetsky, R.; Levdansky, L.; Ringel, I. and Vexler, A. (1998). Paclitaxel-induced modification of the effects of radiation and alterations in the cell cycle in normal and tumor mammalian cells. Radiat Res., 150(3): 283-291.