Vol 5 Issue 9 Oct 2015

ISSN No : 2230-7850

International Multidisciplinary Research Journal

Indian Streams Research Journal

Executive Editor
Ashok Yakkaldevi

Editor-in-Chief
H.N.Jagtap

Welcome to ISRJ

RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

Indian Streams Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial board.Readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

International Advisory Board

Editorial Board

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.org

Indian Streams Research Journal
ISSN 2230-7850

Impact Factor : 3.1560(UIF)
Volume - 5 | Issue - 9 | Oct - 2015

GENOTOXIC EFFECT OF TAMOXIFEN AS AN ANTI-CANCER DRUG

First Author Details :

Gehan M. Ibraheim
Molecular Biology Dept., Genetic Engineering & Biotechnology Reseach Institute (GEBRI), Sadat City University, Sadat City, Egypt.

Co - Author Details :

Hadia A. Heikal and Doaa Amer
Molecular Biology Dept., Genetic Engineering & Biotechnology Reseach Institute (GEBRI), Sadat City University, Sadat City, Egypt.

ABSTRACT

The purpose of this study was to evaluate the cytogenetic damage induced by tamoxifen (z-1,2-diphenyl-1-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-butene) via determining the percentages of chromosomal aberrations from rat bone-marrow cells and human leucocytes, percentages of polychromatic erythrocytes (PCE) cells in rat and DNA profiling extracted from rat liver. The results indicated that there were three main different types of aberrations (deletions, fragments and stickiness) in both rat bone-marrow and human leucocytes, whichshowed that the aberrant metaphase total percentages (11, 18 and 27%) and (10, 21 and 32%) were increased by increasing the tamoxifen dose (0.1, 0.15 and 0.2µg/kg) and (2, 5 and 10 µg/ml), respectively. Stickiness aberrationwerethe highest percentages (6, 7 and 10%) and (4, 9 and 13%) with doses (0.1, 0.15 and 0.2 µg/kg) and (2, 5 and 10 µg/ml) from rat bone-marrow and human leucocytes, respectively. The total percentages of micronucleated polychromatic erythrocytes (0.43, 0.55 and 0.76%) were also increased by increasing tamoxifen dose (0.1, 0.15 and 0.2 µg/kg), respectively. On the other hand, DNA rat liver showed one band with control, treatment2 (T_2) and treatment3 (T_3) (810, 480 and 810 kbp), respectively, whereas treatment1(T_1) gave two bands (777 and 472 Kbp). This demonstrated that the high percentage of aberrations at the high concentration of tamoxifen may be caused by its capability to interfere with spindle fiber or formed DNA adduct. In conclusion, we can confirm that, tamoxifen is capable to cause damage in genetic material according to its dose and it may due to some reasons such as: the ability of cells to repair the damage; cell killing effect and inactivation of drug or its metabolites.

KEYWORDS :*Chromosomal aberrations, DNA, genotoxicity, human leucocytes, polychromatic erythrocytes (PCE), rat, tamoxifen.*

INTRODUCTION:

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. It caused by external factors like (tobacco, infectious organisms and an unhealthy diet)

and internal factors (inherited genetic mutations, hormones and immune conditions) (American Cancer Society, 2015). Breast cancer is the most common cancer in women worldwide and the second most common cancer overall. It is the most frequently diagnosed cancer among women in 140 of 184 countries worldwide. Globally, breast cancer now represents one in four of all cancers in women. Since 2008,worldwide breast cancer incidence has increased by more than 20%,mortality has increased by 14% (Ferlayet al., 2012).

Tamoxifen is a synthetic anti-estrogen used as a medication in pill form that interferes with the activity of estrogen (a female hormone), which promotes the growth of breast cancer cells (Love et al., 1992). Tamoxifen has been used for more than 20 years to treat patients with advanced breast cancer. The drug slowsdown or stops the growth of cancer cells that are present in the body. As adjuvant therapy, tamoxifen helps to prevent the original breast cancer from returning and also helps to prevent the development of new cancers in the other breast(Brown, 2009).

As tamoxifen is a relatively important drug, the full long term effects of the drug are still being assessed through patient studies. In recent years there has been publicity about the long term effects of tamoxifen and its possible relation with other types of cancer (Gelmon, 2000 and Novotny et al., 2000).Data from large treatment studies suggested that there was a small increase in the number of blood clots in women taking tamoxifen, particularly in women receiving anticancer drugs (chemotherapy) along with tamoxifen (Sellman 2010).Bone-marrow cytogenetic analysis was extended to evaluate drug toxicity by several inverstigators (Tateset al., 1977; Van Buual and Gougzwaard, 1980; Temtamyet al., 1982 and Reddy, 1984). Many scientists reported the induction of micronuclei in mice bone-marrow PCE by mutagens, drug, pesticides and plant flavonoids (Matter and Schmid, 1971, Matter and Grauwiler, 1974; Chaubeyet al., 1978; Sahuet al., 1981 and Yamamoto and Kikuchi, 1981).

The present study aims to investigate the genotoxicity of tamoxifen in rats and human using evaluation of aberrations in rat bone-marrow chromosomes, micronucleus test of polychromatic ratserythrocytes (PCE), DNA rat liver, and chromosomal abnormalities in human leucocytes.

MATERIALS AND METHODS

Materials:

Fisher rats, used in this study, were obtained from Faculty of Veterinary Medicine, University of Sadat City, Egypt.

Methods:

Four groups of rats were used, each consisting of three animals; three groups for treatments and the fourth group as a negative control, which had been given the appropriate volume of deionized water.Three selected doses of the tamoxifen drug (0.1, 0.15 and 0.2 µg / kg) were applied orally once daily for 10 days, since 3 animals were used for each dose. After 24 hours of the 10th day, the animals were slaughtered. The animals were injected with 0.6 mg / kg of colchicine 3 hours before slaughtering the rats. After slaughter, the adhering soft tissue and epiphysis of both tibiae were removed.

Analysis of chromosomal abnormalities in rat bone-marrow cells:

Part of the bone marrow was transferred to phosphate saline buffer and centrifuged at 2000 rpm for 5 min. The pellet re-suspended in 0.075 M KCl, and then re-centrifuged and the pellet suspend again in fixative solution (methanol: acetic acid by 3: 1). The fixative solution was changed after two hours and the cell suspension was stored at 4°C overnight,after that, dropped on very clean glass slides

and air-dried (Brusick, 1980).

Analysis of micronucleus test of polychromatic erythrocytes rats:

Bone marrow processing was done according to Schmid (1975), where part of the bone marrow was transferred to a centrifuge tubes containing 5 ml of fetal calf serum (tube for each animal), and centrifuged at 1000 rpm for 5 min. The supernatant was eliminated and the pellet was transferred on the end of the slid and spread by a cover glass held at an angle of about 45º, and then air-dried.

Staining:

In the case of chromosomal abnormalities in rat bone marrow 10% Giemsa at pH 6.8 were used. Whereas, micronucleus test polychromatic erythrocytes in rat were stained in ordinary vertical staining jar according to Gollapudi andKamra (1979). The slides were fixed in absolute methanol for 10 min, then stained in Giemsa (1: 6 Gurr's R-66 Giemsa in deionized water) and placed again in deionized water, air-dried and cleaned in xylene for 3 min.

Screening of slides:

Analysis of chromosomal abnormalities in rat bone marrow:

Slides were coded and screened for chromosomal abnormalities such as: gaps, deletions, fragment breaks, stickiness and polyploidy. A mitotic index based on at least 1000 counted cells was recorded. For chromosomal abnormalities at least 200 metaphase cells per dose were recorded, then compared with the negative control and statistically analyzed.

Micronucleus test analysis of polychromatic erythrocytes in rat:

In this study, a thousands of polychromatic erythrocytes per animal were scored according toBrusick (1980) using a manual counter. The frequency of micronucleated cells has been expressed as a present of micronucleated cells based on the total polychromatic erythrocytes percent.Micronucleoli were identified as dark blue stained bodies in the cytoplasm of polychromatic erythrocytes. The data obtained from this study were analyzed according to Hart and Pedersen (1983).

Analysis of chromosomal abnormalities in human leucocytes:

To investigate the ability of tamoxifen anti-cancer drug in causing chromosomal abnormalities in human karyotype, heparinized venous blood were collected from normal healthy adults and cultured on a standard leucocyte culture (Dean &Denford, 1974) consisted of an minimum essential medium (MEM) (eagle) with L-Glutamine (SIGMA) supplemented with 10% fetal calf serum (SIGMA) and 1% penicillin: streptomycin (1000 units: 1000 micrograms / ml). Per 10 ml of this medium, 0.5 ml whole blood and 0.25 ml phytohemagglutinin (GIBCO) were added. The cultures were incubated in tightly sealed tubes at 37º C for 72 hours. The three doses of tamoxifen drug were added after 62 hours of incubation (10 hours before harvesting). Two hours prior to harvesting, 0.1 ml colcemid (GIBCO) was added for each culture and re-incubated for two hours at 37º C.

Metaphase chromosomes were prepared according to Schwarzacher (1974) as follows: The cultures were centrifuged for 8 min at 1200 rpm, the supernatant was discarded and the cell pellet re-dissolved with the last point of the supernatant, then adding 8 ml of pre-warmed (37º C) hypotonic solution (0.5 M KCl), and left for 10 min at 37º C, after that centrifuged for 8 min at 1200 rpm, then fixed for an hour in about 8 ml freshly fixed fluid (3 parts methanol : 1 part Glacial acetic acid) and centrifuged. The cell pellet was fixed 3 times for 10 min each.Human chromosomes were stained using

10% Geimsa and the chromosomes were investigated for the presence of breaks, gaps and deletions.... etc.

DNA extraction and electrophoresis:

Genomic DNA of rat liver was extracted using ferments kit. Extracted DNA was electrophoresed using 1% ethidium bromide stained agarose gel with 0.5X Tris-Borate-EDTA (TBE) buffer. Obtained DNA bands were visualized using UV-transilluminator and immediately photographed using digital Camera (Fuji 100) with orange filter (Life Science Group, 2000).

RESULTS

Rat bone-marrow chromosomes:

The analysis of rat bone-marrow cells are given in Table (1) and Figure (1). In this experiment 200 cells were analyzed for each treatment. Cytological examination revealed that, there were no aberrant metaphases in the negative control group (Fig., 2), while three main different types of aberrations (deletions, fragments and stickiness) were observed (Fig., 3). The total percentages of aberrant metaphase were 11, 18 and 27 with doses 0.1, 0.15 and 0.2 µg/kg, respectively. However, stickness represented the highest percentages (6, 7 and 10%) of the aberrant metaphases of the applied doses 0.1, 0.15 and 0.2 µg/kg b.wt., respectively. The results revealed that, deletions (2, 6 and 9%), fragments (3, 5 and 8%) and stickness (6, 7 and 10%) were increased by increasing the drug dose (0.1, 0.15 and 0.2 µg/kg), respectively.

Table 1: Chromosomal aberrations of rat Bone-marrow after treatment with tamoxifen.

Dose (µg/kg)	Percent of metaphases with			
	Chromosome deletion	Fragment	Stickiness	% Aberrant metaphase
Control	-	-	-	-
0.1	2	3	6	11
0.15	6	5	7	18
0.2	9	8	10	27

(200 cells were analyzed).

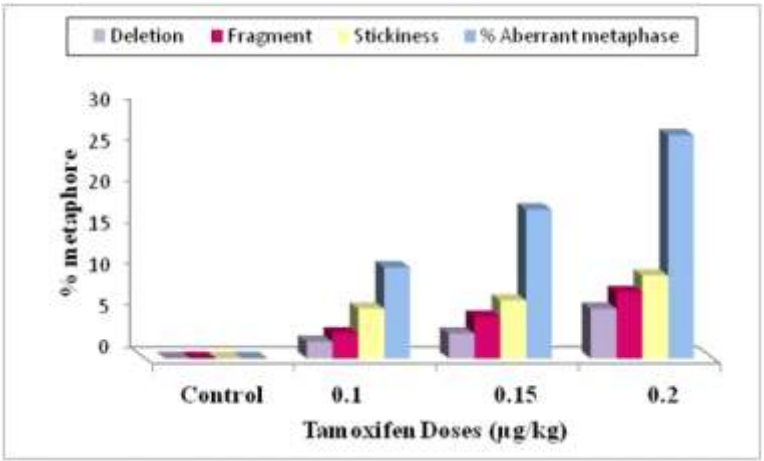

Fig. 1: Chromosomal aberrations of rat Bone-marrow after treatment with tamoxifen.

Fig. 2: Photomicrograph showing untreated rat chromosomes.

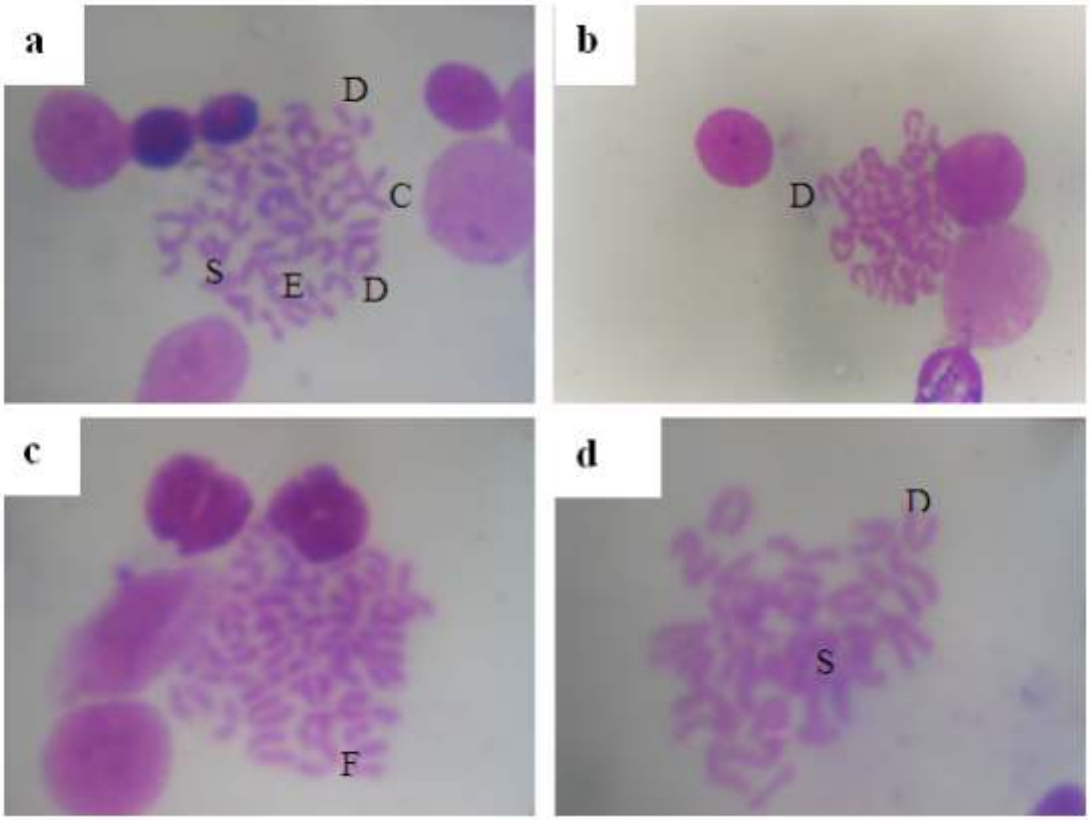

Fig. 3: Photomicrograph of rat chromosomes after treatment with Tamoxifen.
(C: centric fusion; D: deletion; E: end to end association; F: fragment; S: stickiness)

MICRONUCLEUS TEST

The analysis of micronuclei in rat polychromatic (immature) erythrocytes (PCE) after treatment for 10 days with antibreast cancer drug is shown in Table (2) and presented in Figs. (4-6).Normochromatic erythrocytes (NMCs) were omitted. The total PCE counted were 400 for control as well as for the different treatments investigated. The least PCE with micronuclei was detected at the control test. However, by increasing the dose, PCE with micronuclei increased gradually till it reached the maximum value (31) at the highest tamoxifen dose (0.2 µg/kg). The average percentages of micronucleatedPCE induced by Tamoxifen pretreatment were 0.43, 0.55 and 0.76 for the used doses

0.1, 0.15 and 0.2 µg/ kg b. wt., respectively, while that of the control group was only 0.18% (Fig.4).

Table 2: Polychromatic erythrocytes (PCE) cells in rat after treatment with tamoxifen.

Dose (µg/kg)	Total PCE counted	PCE with micronuclei	% of PCE with micronucleus
Control	400	6	0.18
0.1	400	17	0.43
0.15	400	22	0.55
0.2	400	31	0.76

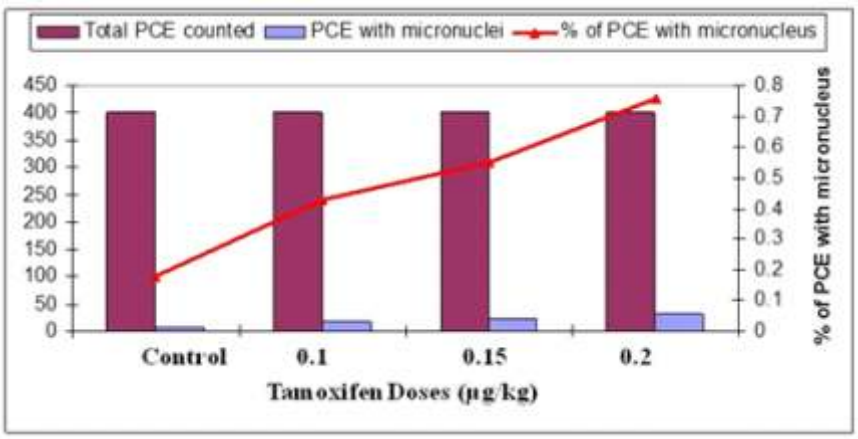

Fig. 4: Polychromatic erthrocytes (PCE) cells in rat after treatment with tamoxifen.

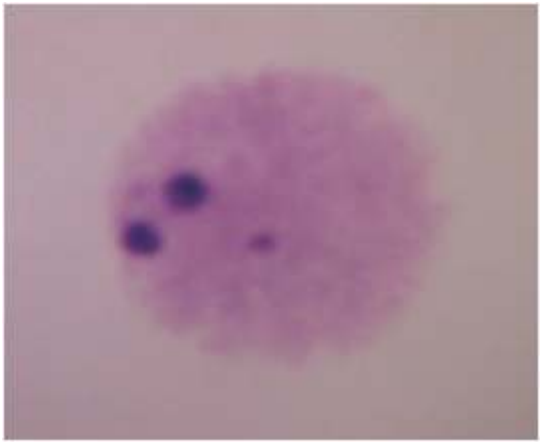

Fig. 5: Polychromatic erythrocytes in rat bone marrow (Control).

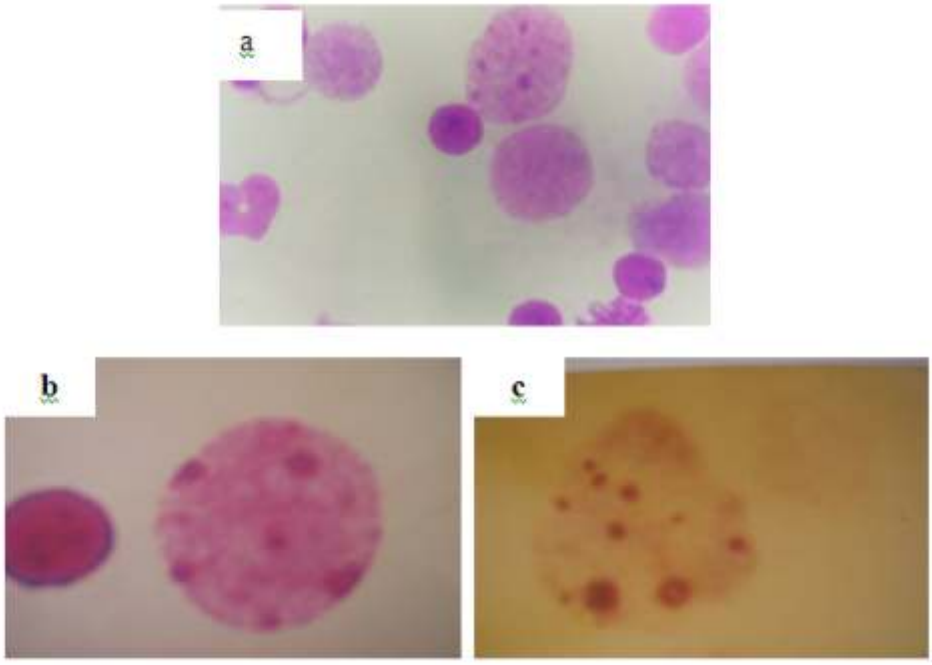

Fig. 6: Photomicrography showing micronuclei in rat, each one with multibodies after treatment with tamoxifen.

Analysis of chromosomal abnormalities in human leucocytes

Cromosomal abnormalities in human leucocytes after treatment with different concentrations of Tamoxifen drug has been shown in Table (3) and Fig. (7). Results revealed that, there were three main types of chromosomal aberrations, deletions, fragments and stickiness. The percentages of aberrant metaphase were 10, 21 and 32% induced by tamoxifen concentrations 2, 5 and 10µg/ml, respectively, which were increased by increasing the dose. One the other hand, comparing the normal human karyotype with those obtained after treatment with tamoxifine drug doses,showed that the normal chromosome morphology was altered.

Table 3: Chromosome abnormalities induced after treatment of human leucocytes with tamoxifen.

Dose (µg/ml)	Fragment	Deletion	Stickiness	% of aberrations
Control	0	1	1	2
2.0	2	4	4	10
5.0	5	9	7	21
10.0	7	12	13	32

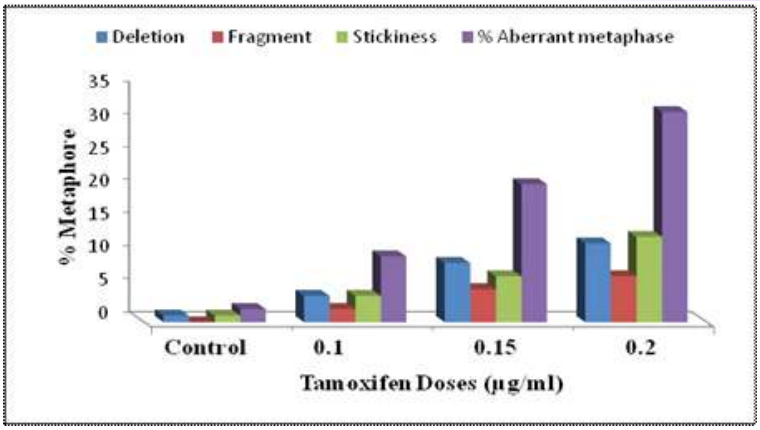

Fig. 7: Chromosome abnormalities induced after treatment of human leucocytes with tamoxifen.

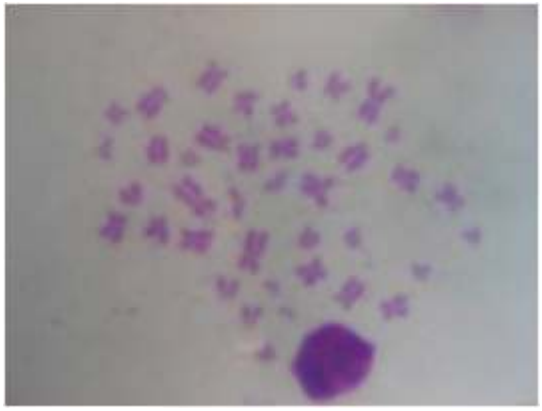

Photo 8: Photomicrograph showing untreated human chromosomes.

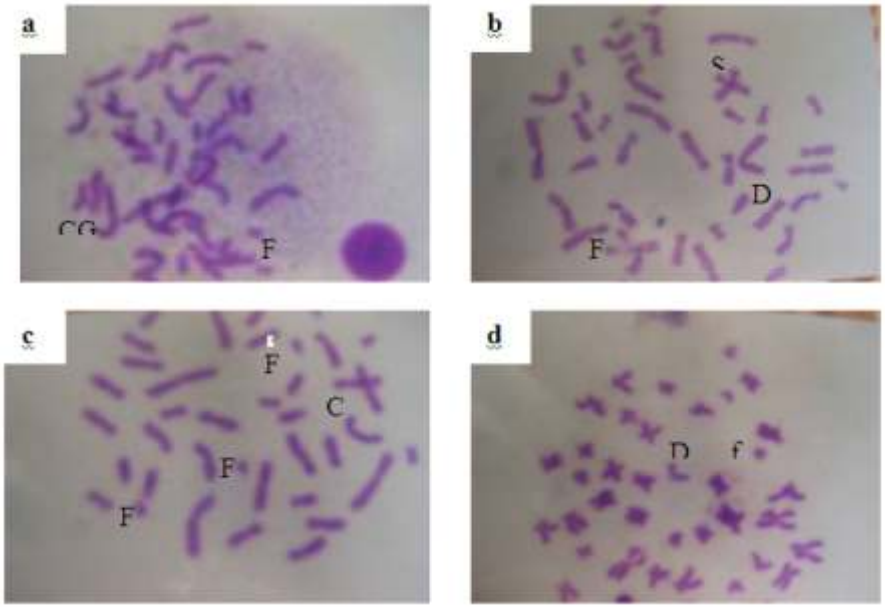

Fig. 9: Photomicrograph of human chromosomes after treatment with Tamoxifen.
(C: centric fusion; D: deletion; CG: Chromatid gap; F: fragment; S: sticky)

Examination of Rat liver DNA using agarose gel electrophoresis:

Agarose(1%)gel electrophoresis for extracted rat liver DNA was shown in Fig. (10). It indicates that there were differences between total DNA from control bone marrow and from treated animals. Fig. (10) showed that control, T2 and T3 showed one band with molecular weights 810, 480 and 810Kbp, respectively, whereas T1 showed 2 bands with molecular weights, 777 and 472 Kpb.

Fig. 10:Photomicrograph showing DNA content in rat liver cells treated with different concentrations of Tamoxifen (M= marker, T_1= 2µg/ml, T_2= 5 µg/ml, T_3= 10 µg/ml) as well as control(C)samples.

DISCUSSION

The results of the present study indicated that the use of tamoxifen is associated with induction of chromosomal abnormalities. The total percentages of aberrant metaphase (11, 18 and 27%) and 10, 21 and 32%) were increased by increasing the tamoxifen dose (0.1, 0.15 and 0.2 µg/kg) and (2, 5 and 10 µg/ml) with rat bone-marrow and human leucocytes chromosomes, respectively. the maximal effects of tamoxifen on abberations are stickiness (6, 7 and 10%) and (4, 9 and 13%) with doses (0.1, 0.15 and 0.2 µg/kg) and (2, 5 and 10 µg/ml) in rat bone-marrow and human leucocytes, respectively. It probably seems that the high percentages of aberrant metaphases might be caused by cytoplasmic disturbance induced by high concentrations.

On the other hand, the results indicated that the used doses (0.1, 0.15 and 0.2 µg/kg) of tamoxifen induced total percentages of micronucleated PCE (0.43, 0.55 and 0.76%), respectively, and increased by increasing the tamoxifen dose.Results gave evidence that the drug is capable to interfere with spindle fibers.

Gotezet al. (1975) found a clear cut that cyclophosphamide dose effect relationship in the frequency of breaks as well as abnormal metaphase. Reddy (1984) studied the effect of paracetamolon mouse bone-marrow chromosomes and observed chromosomal anomalies such as gaps, breaks, fragments and polyploidy nuclei. Van Buul and Goudzwaard (1980) found that the antibiotic bleomycin induced structural chromosomal aberrations in spermatogonia as well as bone-marrow cells of mice. Martinez-Climentet al. (2000) determined prospectively the incidence of chromosomal abnormalities

in patients with high-risk breast cancer after high-dose chemotherapy. Hakeem and Amer (1965) concluded that the common effects of steroid hormone on chromosomes are stickiness in diakinesis metaphase, and bridges and sometimes lagging chromosomes in the anaphase. The in vitromicronuclei test (MNT) allows the detection of breakage, chromosome loss and non-disjunction (Parry and Sorrs, 1993 and Kirsch-Volders, 1997). Bonassiet al. (2007) gave the preliminary evidence that micronuclei (MN) frequency in peripheral blood lymphocytes (PBL) is a predictive biomarker of cancer risk within a population of healthy subjects.

At level of rat liver DNA profile investigation, the results showed that control and T3showed one band with the same molecular weight (810 kbp), whereas T2 gave one band also, but with lower molecular weight (480 Kbp). On the other hand, T1 showed two bands with molecular weights 777 and 472 kbp. It may be caused by one or all of the following reasons: i) overdose of the drug, which may cause changes of DNA configuration; ii) some fragments founded; and iii) repairs of damaged genetic material. Davis et al. (2000) concluded that tamoxifen causes mutations in the livers of transgenic rats, further strengthens the causal link between DNA adduct formation and tumorigenicity of this compound. The presence of tamoxifen-induced DNA adducts (Han and Liehr, 1992; White et al., 1993; and Styles et al., 1994) may result in premature condensation, exchanges between chromosomes, and chromosomal breakage. Di Paolo and Popescu (1977) indicated that tamoxifen-induced DNA damage causes changes in cell cycle progression and further karyotypic instability.

In conclusion, the present study demonstrated that the use of tamoxifen is associated with chromosomal aberrations and the maximal effect was observed at the highest concentration and the common effects of tamoxifen on chromosomes are stickiness.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. HanafiHamza, Prof. Microbiology, Genetic Engineering & Biotechnology Research Institute, Sadat City University, Egypt for his contribution, great help and his constructive comments on the manuscript. We would also like to thank Dr. Mohammed Othman, Prof. Bioinformatics, Genetic Engineering & Biotechnology Research Institute, Sadat City University, Egypt for his contribution in the preparation of this work.

REFERENCES

1.American Cancer Society 2015. Cancer facts & figures 2015. Atlanta: American Cancer Society 2015. pp. 1-2.

2.Bonassi, S., Znaor, A., Ceppi, M., Lando, C., Chang, W.P., Holland, N., Kirsch-Volders, M., Zeiger, E., Ban, S., Barale, R., Bigatti, M.P., Bolognesi, C., Cebulska-Wasilewska, A., Fabianova, E., Fucic, A., Hagmar, L., Joksie, G., Martelli, A., Migliore, L., Mirkova, E., Scarfi, M., Zijno, a., Norppa, H., and Fenech, M. 2007. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in human. Carcinogenesis. 82(3): 625-631.

3.Brown K. 2009. Is tamoxifen a genotoxic carcinogen in women? Mutagenesis. 24(5): 391-404.

4.Brusick, D. 1980. Principles of genetic toxicology. Plenum Press; N.Y., pp. 279.

5.Chaueby R.C., Kavi B.R., Chauhan P.S., and Sandaram K. 1978. The effect of hycantone and maleic hydrazide on the frequency of micronuclei in the bone marrow erythrocytes of mice. Mutat. Res. 57: 187-191.

6.Davis W., Hewer A., Rajkowski K.M., Meini W., Glatt H. and philips D.H. 2000. Sex differences in the activation of tamoxifen to DNA pinding species in rat liver in vivo and in duction. Cancer Res. 60: 287-289.

7.Dean, B.J. and Denford, N. 1974. Assays for the detection of chemically-induced chromosome damage in cultured mammalian cells. In: Mutagenicity testing; a practical approach (Eds. S. Venitt& J.M. Parry) IRL Press.
8.Di Paolo, J. and Popescu 1977. Banding pattern analysis of initial structural chromosome alterations induced by N-methyl-N-nitroso-N-nitrosoquinone in syrian hamster cells. Mutat. Res. 44: 359-368.
9.Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., Globocan, B.F. 2012. Cancer incidence and mortality worldwide. IARC Cancer Base No. 11; IARC Publisher.
10.Gelmon K. 2000. One step forward or one step back with tamoxifen?Lancet.356(9233): 868-869.
11.Gollapudi, B. and Kamara, O.P. 1979. Application of a simple Gimesa-staining method in the micronucleus test.Mutat. Res., 64: 45-60.
12.Gotez P., Sram R.J. and Dohanlova J. 1975. Relationship between experimental results in mammals and man. I. cytogenetic analysis of bone marrow injury induced by a single dose of cyclophosphamide. Mutat. Res. 31: 247- 257.
13.Hakeem, H. and Amer, S. 1965. Meiotic effects of water extracts of some contraceptive tablets. Naturwissenschaften, 53(7): 184-184.
14.Han, Y. and Liehr, J.G. 1992. Induction of covalent DNA adducts in rodents by tamoxifen. Cancer Res. 52: 1360-1363.
15.Hart, J.W. and Pederson, E.H. 1983. Statistics of the mouse bone-marrow micronucleus test: counting, distribution and evaluation of results. Mutat. Res., 111: 195-207.
16.Kirsch-Volders M. 1997. Towards a validation of the micrnuclei test. Mutat. Res., 392: 1-4.
17.Life Science Group 2000. Sub-cell® GT agarose gel electrophoresis system: instruction manual. Catalog Numbrs: 170-4401 to 170-4406 and 170-4481 to 170-4486.
18.Love R. Richard, R.B. Mazess, H. S. Barden, S. Epstein, P. A. Newcome, V.V. Jordan, P.P. Carbone and D.L. Demets 1992. Effect of Tamoxifen on bone mineral density in postmenopausal woomen with breast cancer. The new England J. Medicine, 326(13): 852-856.
19.Marteniez-Climent J.A., Comes A.M., Vizcarra E., Benet I., Arbona C., Prosper F., Solano C., Garcia Clavel B., Marugan I., liuch A. and Garcia-Conde J. 2000. Chromosomal abnormalities in women with breast cancer after autologous stem cell transpianation are infrequent and may not predict development of therapy-related leukemia or myelodysplastic syndrome. J. NPG. Bone marrow transpianation, 25(11):1203-1208.
20.Matter B.E. and Grauwiler J. 1974. Micronuclei in mouse bone marrow cells. A simple in viva model for the evaluation of drug-induced chromosomal aberrations. Mutat. Res. 23: 239-249.
21.Matter B.E. and Schmid W. 1971. Trenimon induced chromosomal damage in bone marrow cells of six mammalian species, evaluated by the micronucleus test. Mutat. Res. 12: 417-425.
22.Novotny L., Rauko P., Vachálková A., Peterson-Biggs M.2000. Tamoxifen in cancer therapy: minireview.Neoplasma. 47(1):3-7.
23.Parry J.M. and Sorrs A. 1993. The detection and assessment of the aneugenic potential of environmental chemicals: the European community Aneuploidy project Mutat. Res. 287: 3- 15.
24.Reddy G. A. 1984. Effect of paracetamol in chromosome of mouse bone marrow. Cariologia. 37: 127-132.
25.Sahu R.K., Basu R. and sharma A. 1981. Genetic toxicological testing of some plant flavonoids by the micronucleus test. Mutat. Res. 89: 69-74.
26.Schmid, W. 1975. The micronucleus test. Mutat. Res. 31: 9-15.
27.Schwarzacher, H.G. 1974. Preparation of metaphase chromosomes. In: Methods in human

cytogenetics (eds. H.G. Schwarzacher& U. Wolf), Berlin.
28.Sellman S. 2010. Beware of the Dark Side ofTamoxifen (Nolvadex). Optimal Health Products: Offers Cruciflax, a natural alternative to tamoxifen, which is a whole food supplement that eliminates toxic estrogen metabolites from the body. www.blisscream.com. 888: 641-2547.
29.Styles, J.A., Davies, A., Lim, C.K., de Matteis, F., Stanley, L.A., White, I.N.H., Yuan, Z.Z., Smith, L.L. 1994. Genotoxicity of tamoxifen, tamoxifen epoxide and toremifene in human lymphoblastoid cells containing human cytochrome P450s. Carcinogenesis. 15: 5-9.
30.Tates A.D., Natarajan A. T., Devogel N. and Meljers M. 1977. A correlative study on the genetic damage induced by chemical mutagens in bone marrow and spermatogonia of mice. III - 1,3-Bis C 2-chloroethyl-3-nitrosourea (BCNU). Mutat. Res. 44: 87-95.
31.Temtamy, S.A., Dehondt and M.A. El –Ghor. 1982. Effect of novalgin on chromosomes of RattusnorvegicusEgypt. J. Genet. Cytol., 11: 105-111.
32.Van Buul P.P.W. and Goudzwaard J.H. 1980. Bleomycin induced structural chromosomal aberrations in spermatogonia and bone marrow cells of mice. Mutat. Res. 69: 319-324.
33.White, I.N.H., de Matteis, F., Davies, A., Smith, L.L., Crofton-Sleigh, C., Venitt, S., Hewer, A., Phillips, D.H. 1993. Genotoxic potential of tamoxifen and analogues in female Fischer F344/N rats, DBA/2 and C57BL/6 mice and human MCL-5 cells. Carcinogenesis. 13: 2197-2203.
34.Yamamoto K.I. and KiKuchi Y. 1981. Studies on micronuclei time response and on the effects of multiple treatments of mutagens on induction of micronuclei. Mutat. Res. 90: 163-173.

Publish Research Article
International Level Multidisciplinary Research Journal
For All Subjects

Dear Sir/Mam,

We invite unpublished Research Paper,Summary of Research Project,Theses,Books and Book Review for publication,you will be pleased to know that our journals are

Associated and Indexed,India

- International Scientific Journal Consortium
- OPEN J-GATE

Associated and Indexed,USA

- Google Scholar
- EBSCO
- DOAJ
- Index Copernicus
- Publication Index
- Academic Journal Database
- Contemporary Research Index
- Academic Paper Databse
- Digital Journals Database
- Current Index to Scholarly Journals
- Elite Scientific Journal Archive
- Directory Of Academic Resources
- Scholar Journal Index
- Recent Science Index
- Scientific Resources Database
- Directory Of Research Journal Indexing

Indian Streams Research Journal
258/34 Raviwar Peth Solapur-413005,Maharashtra
Contact-9595359435
E-Mail-ayisrj@yahoo.in/ayisrj2011@gmail.com
Website : www.isrj.org