

Tamoxifen- Induced Chromosomal Aberrations in Pregnant Female Rats

Ramadan A.M. Ali*; Mohamed A. Rashed; Mervat M. Labib*; Sara H. Fawzy.***

***Zoology Dept., Faculty of Women for Arts, Science and Education, Ain -Shams University.**

**** Genetics Dept., Faculty of Agriculture, Ain-Shams University.**

Abstract

Tamoxifen is a non-steroidal, selective estrogen receptor modulator (SERM) used in the treatment of all the stages of breast cancer patients at all ages. Recent evidences showed that tamoxifen could induce cancers which might be a consequent result for chromosomal aberrations in rats. For investigating the chromosomal aberrations induced by tamoxifen treatment, pregnant female rats (four rats per treatment) received tamoxifen orally at dose 0 and 20 mg/ kg b. wt. at different periods. Animals were injected (ip.) with colchicine at 1.5 hr prior sacrifice. Rats were sacrificed 24hr after last treatment. bone marrow cells where collected, then the cells were prepared for karyotype evaluation. 50 well spread metaphase plates were examined per animal. Tamoxifen induced significant increases in the frequencies of structural chromosomal aberrations, like gaps, Centric fragments, dicentric chromosomes, centric separation and centric fusions in the bone marrow metaphases. However, tamoxifen did not elevate the averages of numerical chromosomal aberrations than the averages of control level. Moreover, a significant increase in the mitotic activity of the bone marrow cells was observed after tamoxifen treatment. The present study proved the clastogenic and the spindle poisoning action of tamoxifen. Consequently, risks against benefits should be carefully evaluated when tamoxifen is used as a therapy of choice on humans.

Key words: tamoxifen, chromosomal aberrations, bone marrow, rat, pregnancy.

1. Introduction

During the 1960's, Imperial Chemical Industry (ICI) (now called AstraZeneca) at Alderley Park realized the anti-fertility and the anti-estrogenic effects of a new compound called triphenylethylene ICI 46,464 (**Harper and Walpole, 1967**). Triphenylethylene ICI 46,464 is now sold under the trade names Tamoxifen, Nolvadex, Istubal, Valodex and Genox.

Tamoxifen was firstly used for treatment of post-menopausal breast cancer patients (**Peto, 1989**). Tamoxifen (TAM) a triphenylethylene anti-estrogen reduces the incidence of breast

corresponding author: Sara.hatem@women.asu.edu.eg

cancer among high-risk women who have never been diagnosed with the disease or with a family inherited breast cancer (**Fisher *et al.*, 1998**). Tamoxifen acts as a mixed agonist/antagonist that prevents osteoporosis in women with breast cancer (**Kim *et al.*, 2002**). It is used for the treatment and prevention of breast cancer in women and it had both estrogenic and non estrogenic effects, depending on the target tissue (**Jordan, 2004 and 2007**).

Tamoxifen therapy might not be recommended during pregnancy, due to the foetal abnormalities caused by its treatment (**Cullins *et al.*, 1994**). Maternal exposure to tamoxifen during pregnancy resulted in abnormalities in the reproductive tract of the offspring (**Diwan *et al.*, 1997**). Animal studies results revealed that maternal exposure to tamoxifen during pregnancy acts as an estrogen in the foetal mammary gland and increases the susceptibility to breast cancer among the female offspring (**Clarke *et al.*, 2000**).

Tamoxifen treatment at the doses of 0.1–0.8 mg/kg/day for 10 days induced chromosomal aberrations in mouse bone marrow cells (**Vijajalaxmi and Rai, 1996**). Tamoxifen also induced a significant increase in the frequency of chromosomal aberrations mainly chromosomal gaps and chromosomal breaks in the cells of the bone marrow and the embryonic cells (**Mokashi *et al.*, 2010**). Tamoxifen showed high levels of DNA adducts in the liver of the treated rats (**Li *et al.*, 1997; Kim *et al.*, 2006**). However, these DNA -adducts are not efficiently repaired from the target tissue which might lead to cancer (**Kim *et al.*, 2006**).

The metabolism of tamoxifen is complex and the major metabolites are 4-hydroxy tamoxifen and N- desmethyl tamoxifen that are not reactive towards DNA. It has been shown that the major mechanism of carcinogenicity of tamoxifen in the rat is through the production of α -hydroxytamoxifen to a genotoxic DNA-binding species (**Boocock *et al.*, 1999**). The carcinogenicity of tamoxifen was evident in human endometrium, and it might be carcinogenic in other tissues, such as the liver and the gut (**Fisher *et al.*, 1994**). **Beland *et al.* (1999)** observed an extensive hepatic DNA adduct are formed by tamoxifen, they stated that these adducts might result from α -hydroxy-tamoxifen and that DNA adducts could be detected in the uterine DNA from rats treated with tamoxifen.

The aim the present study is to investigate the chromosomal aberrations induced by tamoxifen in the bone marrow cells of adult female pregnant rats before mating and during pregnancy. Such genetic damage may reflect the reduction in the reproductive ability of the treated females or affect their offspring.

2. Materials and Methods

2.1. Animals.

Fertile adult virgin female albino rats, purchased from the National Organization for Drug Control And Research (NODCAR) at 6 Abou Hazem St., Pyramids Avenue, Giza, Egypt. The rats were acclimatized for one week at the animal house before the experimentation. The rats were cared humanly and exposed to regular dark/light cycles every 12 hr and good ventilation at temperature 20-22°C. Commercial balanced diet pellets and water were provided *ad libitum*.

2.2. Tamoxifen Exposure.

Tamoxifen is manufactured by AstraZeneca, UK and purchased under the trade name Nolvadex. Tamoxifen is dissolved in dist. sterile water and administered as oral suspension through gastric gavage. The females were treated daily with 20 mg/kg b.wt. of tamoxifen orally.

2.3. Animal Grouping.

Animals were grouped into 4 groups each of 4 females. The first group (control group) included female animals received dist. water daily. Group (2) included fertile female animals received daily oral doses of 20 mg/kg b. wt. for 21 continuous days before mating. The females were then dissected on the 20th day of gestation. Group (3) included pregnant females received oral daily doses of 20 mg/kg b.wt. starting from the 7th day till the 19th day of gestation, then dissected on the 20th day of gestation. Group (4) included pregnant females received oral daily doses of 20 mg/kg b.wt. starting from the 7th day till the 20th day of gestation, and then were dissected after normal delivery.

2.4. Chromosome aberration test

Female rats from the control and the tamoxifen-treated groups were intraperitoneally injected with colchicine 1.5 hr prior to sacrifice (**Preston *et al.*, 1987; Mokashi *et al.*, 2010**). Both femurs were dissected out. Bone marrow cells were collected from the femurs by flushing in test tube containing phosphate buffer solution, centrifuged at 1000 r.p.m. for 10 min at room temperature and then incubated in 5 ml of pre-warmed hypotonic solution (KCl, 0.56%) at 37°C for 35 min. The cell pellet was treated with a fixative formed of in 3 methanol: 1 glacial acetic acid. Centrifugation and fixation were repeated 2 times at an interval of 20 min. The final cell pellet was resuspended in a small volume of the fixative, dropped onto chilled slides, air-dried and stained with 1 % Giemsa solution. The stained

slides were investigated with binocular light microscope with oil immersion lens (100 X). Chromosomal aberrations were spotted among 50 well spread metaphase plates per each animal. Moreover, any deviation in the regular rat chromosome number (diploid number, $2n = 42$ chromosomes) was recorded as numerical aberrations (aneuploidy). Metaphases with extra chromosome(s) were recorded as hyperdiploidy. While, those with missing chromosome(s) were recorded as hypodiploidy.

2.5. Mitotic index

The mitotic index was determined as a measure of cytotoxicity. Mitotic index is counted as the number of dividing cells at any mitotic stage per 1000 cells per animal (Walker, 1954).

2.6. Statistical analysis.

One way ANOVA was performed using SPSS software version 10 to determine the significant difference between the treated groups and the control group, the significance values were calculated at P value ≤ 0.01 .

3. Results and Discussion.

The investigations for chromosomal aberrations were carried out on 50 metaphase spreads derived from bone marrow cells of animals treated with 0 (control group) and 20 mg/kg b. wt. tamoxifen. The chromosomal aberrations were arranged into numerical and structural aberrations. The mitotic indices were counted as the percentage of the dividing cells among 1000 bone marrow cell / animal / group were recorded as represented in table(1).

3.1. Results

3.1.1. Chromosomal aberration assay

Numerical chromosomal aberrations (aneuploid metaphases) appeared in the form of metaphases with chromosomal number less (hypodiploid metaphases) or more (hyperdiploid metaphases) than the $2n$ number that equals to 42 chromosomes (Fig. 1). The hypodiploid metaphases ranged from 7 to 9 metaphases per 200 examined metaphases. The recorded aneuploidy metaphases were less than 10 % and did not have a constant chromosome number. So, it is useless to construct karyotype to determine the frequently missing or extra chromosome(s). In addition, the aneuploid metaphases averages were not significantly different in treated groups than the control group.

Structural chromosomal aberrations were observed in the form of chromosomes with missing arm piece (deletions), acentric chromosomal fragment(a piece of chromosome without centromere), chromatid gap (a chromosome arm with unstained area, and it's width is

less than the chromatid diameter), chromatid break (a chromatid arm with separated unaligned piece), centromeric separation (the two chromatid arms were separated at the centromere region) and dicentric chromosomes (a chromosome appeared as having two centromere regions). The previously mentioned chromosomal aberrations are presented in figure (2).

The results of structural chromosomal aberrations were summarized in table (1) and figures (1 and 2) showed that administration of 20 mg/ kg/ b. wt. of tamoxifen before mating (group 2) induced non- significant difference in the frequencies of average structural chromosomal aberrations in the bone marrow cells compared to the control group (group 1).

Tamoxifen at dose level 20 mg /kg/ b.wt. (Group 3) induced a total of 38 chromosomal aberrations among chromosomes of the bone marrow cells derived from 4 animals with an average of 9.5 ± 1.29 chromosomal aberrations/ 50 metaphase spreads. Such elevation in the averages of chromosomal aberrations was significant ($P \leq 0.01$) when compared with those of the control animals of group (1). The same trend was observed in the animals of group (4), this group evidently had a higher average of chromosomal aberrations than that of group (3). In group (4), tamoxifen induced a significant increase ($P \leq 0.01$) in an average of 11 ± 1.15 chromosomal aberrations / 50 metaphases / animal, when compared with those of the control group (1).

3. 1.2. Mitotic index

From Table (1) and figure (4), a clearly significant reduction ($P \leq 0.01$) in the percentages of mitotic activity of rat bone marrow cells was observed in the tamoxifen treated groups (2), (3) and (4) compared to the control group. Where, the percentages were, 16.4, 10.35, 9.92 and 9.67 in the control group (1), group (2), group (3) and group (4), respectively.

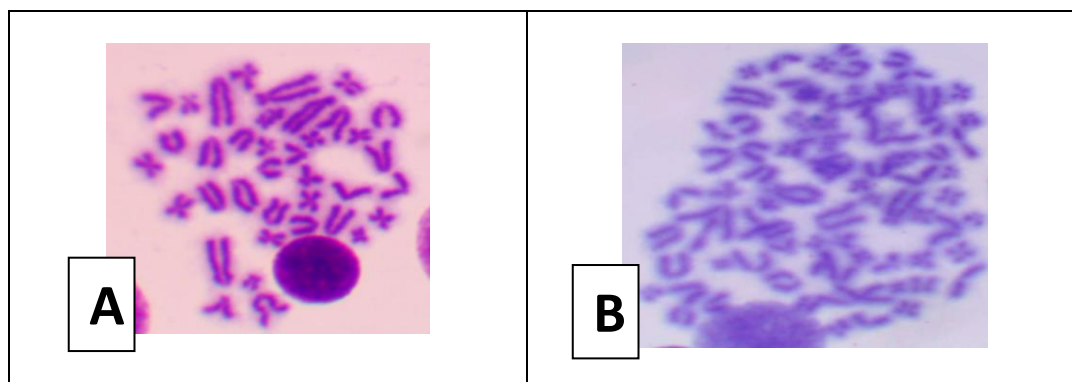


Fig. (1): Aneuploidy metaphases. A: metaphase with less than the 2n chromosomal number and B: metaphase with more than the 2n chromosomal number.

Table (1): Average chromosomal aberrations in metaphase spreads from bone marrow cells of pregnant female rats from the control and the tamoxifen treated groups with 20 mg/kg b.w. tamoxifen at different periods.

Animal Group	Structural Chromosomal Aberrations / 200 Metaphase Spreads							Total Chromosomal Aberrations (Average/ Animal \pm S.D.)	Aneuploid metaphases / 200 metaphase spread		Total Aneuploid metaphases (Average/ Animal \pm S.D.)	% Mitotic Index
	Gap	Break	Centric Fusion	Dicentric	Deletion	Acentric Fragment	Centric Separation		≤ 42	≥ 42		
Group 1	1	1	2	4	2	2	6	18 (4.50 \pm 1.0)	6	2	8 (2.00 \pm 0.82)	16.4
Group 2	2	2	3	5	2	3	4	21 (5.25 \pm 1.5) ^{n.s.}	5	2	7 (1.75 \pm 0.50) ^{n.s.}	10.35**
Group 3	3	2	10	5	5	8	5	38 (9.5 \pm 1.29)***	4	3	7 (1.75 \pm 0.96) ^{n.s.}	9.92**
Group 4	5	3	9	9	5	8	5	44 (11 \pm 1.15)***	6	3	9 (2.25 \pm 1.89) ^{n.s.}	9.67**

N.B.

Group 1 included 4 control animals treated with dis. water.

Group 2 included 4 animals daily treated for 21 before mating and sampling at day 20th of gestation.

Group 3 included 4 animals daily treated for 13 days from the 7th to the 19th days of gestation and sampling at the 20th day of gestation.

Group 4 included 4 animals daily treated for 14 days from the 7th to the 20th days of gestation and sampling after normal delivery.

Statistical comparison was carried out between control and each treated group.

^{n.s.} = non-significant ** = significantly different at ($P \leq 0.01$) *** = significantly different at ($P \leq 0.001$)

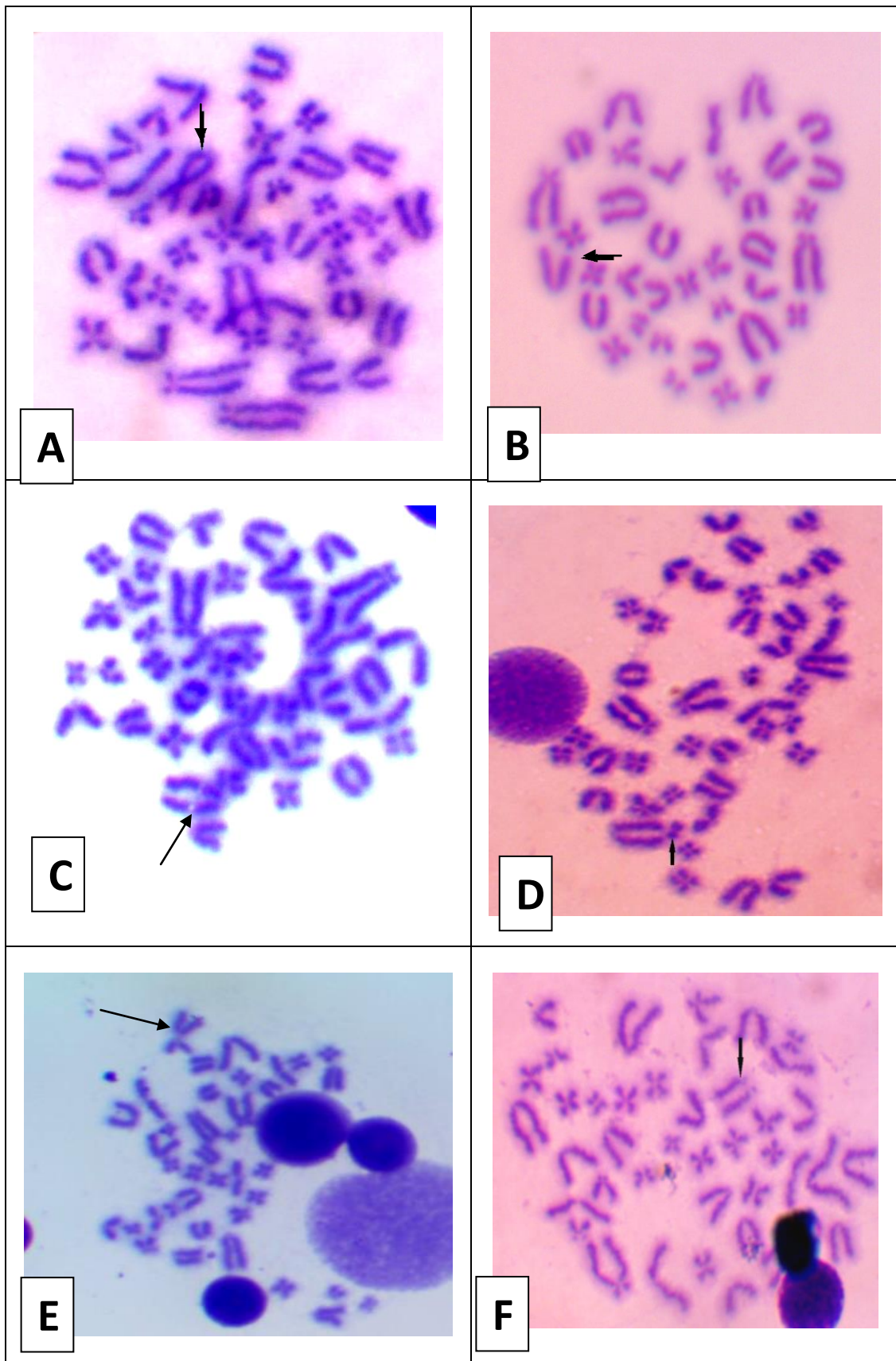


Figure (2): Photomicrographs of metaphase spreads from rats' bone marrow cells from animals of the different treated groups with 20mg/ kg tamoxifen (A) dicentric chromosome, (B) deletion in one of chromosome arms, (C) chromosome gap (D) acentric fragments, (E) centric fusion, and (F) centric separation.

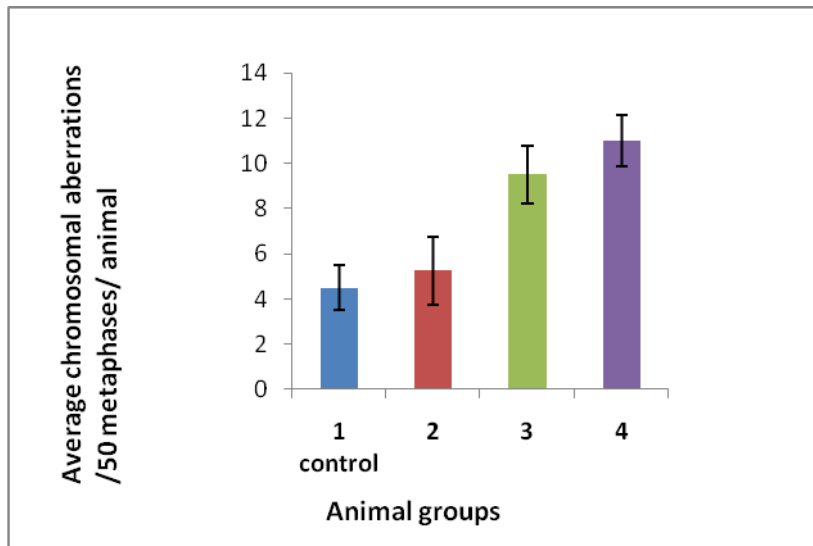


Figure (3): Averages of chromosomal aberrations from rat bone marrow cells from animals of both the control and the treated groups treated with 0 and 20mg/ kg tamoxifen. Group (1) included untreated control animals, group (2) included female rats daily treated for 21 days before mating, group (3) pregnant females treated with 20 mg/ kg/ b.wt. daily from the 7th day of gestation till the 19th day, then dissected at the 20th day of gestation and group (4) pregnant females received 20 mg/ kg/ b.wt. daily tamoxifen from the 7th day till the 20th day of gestation and delivers normally. Note that, the averages of chromosomal aberrations in treated groups increases in comparison with those of control untreated animals of group (1). The averages increase of chromosomal aberrations in animals of group (2) was non-significant, while those of groups (3 and 4) were highly significant ($P \leq 0.001$).

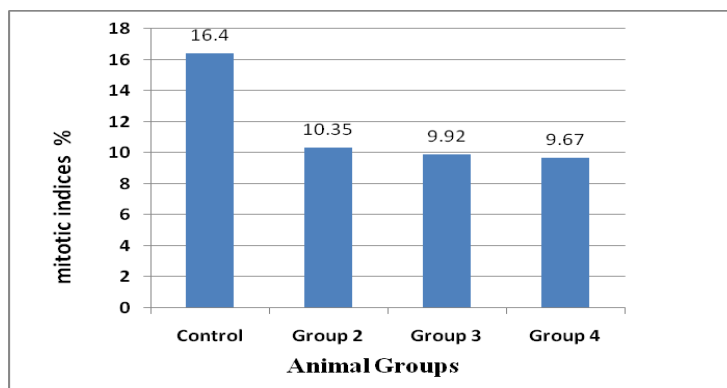


Figure (4) : The mean mitotic indices in the different animal groups. The percentage of mitotic activity significantly ($P \leq 0.01$) decrease in all the treated groups (2, 3 and 4) compared with the control group.

3.2. Discussion

In the present study, the administration of 20 mg/kg/day of tamoxifen for 21 days before mating (group 2) induced non-significant differences in the frequencies of total chromosomal aberrations in bone marrow cells. While, administration of 20 mg/kg/day of tamoxifen during pregnancy starting from the 7th day of gestation (group 3 and 4) induced significant increase ($P \leq 0.001$) in the frequency of total chromosomal aberrations in the bone marrow cells. Statistically significant increase ($P \leq 0.01$) in centric fusion and gaps was observed in the groups treated with tamoxifen during pregnancy. The chromosomal aberrations induced by TAM were previously reported by **Vijayalaxmi and Rai, (1996)**. They indicated that tamoxifen (0.1, 0.2, 0.4 and 0.8 mg/kg/day for 10 days) induced a statistically significant increase in the frequencies of chromosomal aberration in mice bone marrow cells. Moreover tamoxifen could induce structural and numerical alterations in the hepatic chromosomes of rats *in-vivo* (**Sargent et al., 1996; Styles et al., 1997**). Recently, **Yacob and Ismail (2014)** observed chromosomal aberrations in both normal lymphocytes and breast cancer cells in MCF-7 and MCF-10A cell types due to tamoxifen treatment. Tamoxifen also induced micronuclei in human cells and aneuploidy and chromosomal aberrations in rat liver cells (**Phillips, 2001**).

The proposed mechanism for the chromosomal aberration induced by tamoxifen was suggested by (**Sargent et al., 1994; Mokashi et al., 2010**). Which might be due to, the presence of tamoxifen-DNA adducts that causes exchanges between chromosomes, and chromosomal breakage. Tamoxifen is metabolized into an active estrogenic metabolites, that may be activated to reactive metabolite that forms adducts with proteins and DNA (**Destal et al., 2004; Notley et al., 2005; Andersson et al., 2010**). In studies similar to that carries out on the rat, the cynomolgus monkeys were found capable of metabolizing tamoxifen to genotoxic intermediate that form TAM - DNA adducts in various tissues (**Schild et al., 2003; Brown, 2009**). The formation of single and double strand DNA breaks causes oxidation of purines and pyrimidines in these cells that generates free radicals that is thought to be another cause for the chromosomal aberrations (**Yacob and Ismail, 2014**). The tamoxifen is metabolized in the rat's liver into α -hydroxytamoxifen and α -hydroxy-Ndesmethyl tamoxifen that causes cancer (**Pogribny et al., 2007**). The DNA adduct formation constitutes an initial step in the carcinogenic process, as replication of a damaged DNA template can lead to the incorporation of an incorrect base, mutagenesis in critical genes, and a heritable loss of growth control (**Schild et al., 2003**). An important feature of carcinogenic process is a disruption in the balance between cell proliferation and programmed cell death (**Sherr, 2000**).

A significant ($P \leq 0.01$) reduction in the mitotic activity of bone marrow cells of pregnant mother rats were observed after tamoxifen treatment in group (2,3 and 4) compared to the control animal group. This reduction in the mitotic activity due to tamoxifen treatment was investigated by (**Baker *et al.*, 1993**), who reported that compounds structurally similar to tamoxifen blocks the cell cycle progression. In addition to that tamoxifen, induced mitotic arrest (**Otto *et al.*, 1996**) or a cytostatic effects of TAM (**Mandlekar *et al.*, 2000**); this appears to be due to the induction of apoptosis by tamoxifen. Others suggests that the reduction in the mitotic activity might have been due at least two either increased apoptosis that depends primarily on protien synthesis or decreased cell proliferation (**Perry *et al.*, 1995**; **Elis *et al.*, 1997**; **Cameron *et al.*, 2000**).

4. Conclusion

The present study performed on pregnant rats indicates that tamoxifen alters the structure of chromosomes, increases the incidence of chromosomal aberrations. Careful assessment of the risks as well as the benefits of chronic tamoxifen administration should be considered in using this drug during pregnancy. The relatively high frequency of foetal congenital abnormalities indicated by previous studies suggests that a reliable birth control method must be taken during tamoxifen treatment. Also, mothers who are planing to become pregnant must stop tamoxifen treatment for a recovery period.

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الملخص باللغة العربية

التغيرات الكروموسومية الناتجة عن المعالجة بالتاموكسيفين في إناث الجرذان الحوامل.

رمضان احمد محمد علي. * محمد عبدالسلام راشد** . مرفت محمد لبيب الجندي* . ساره حاتم محمد فوزي*.

* قسم علم الحيوان- كلية النبات -جامعه عين شمس.

** قسم الوراثة- كلية الزراعة- جامعه عين شمس.

التاموكسيفين من المواد غير الستيرويدية، وهو يعد من مستقبلات الايستروجين الاختيارية المتغيرة التي تستخدم في علاج اورام الثدي في مراحله المختلفة. و قد اكتُشف مؤخرًا أن التاموكسيفين يؤدي إلي أورام قد تكون ناتجة عن تغيرات في الكروموسومات في الجرذان البيضاء. و في دراسة هذه التغيرات الكروموسومية الناتجة من المعالجة بالتاموكسيفين , تم تقسيم أمات الجرذان الحوامل (أربعة في كل مجموعة) و معالجهم بـ 20 ملج/ كج يومياً في اوقات زمنية مختلفة. وفي اليوم الأخير للمعالجة, تم تشريح الامهات في اليوم التالي. حيث أنه يتم حقن الجرذان قبل تجربته بـ ساعه و نصف داخل الصفاق و معالجتهم بالكولشيسين, ثم يتم تجميع خلايا نخاع العظم و تجهيزها للفحص الكروموسومي. وقد فحص خمسين خلية في الطور الاستوائي لكل حيوان. و كانت النتائج أنه يوجد فروق معنوية في التغيرات الكروموسومية مقارنة بالمجموعه غير المعالجه. كما أنه كانت هناك فروق معنوية بالنقصان للنشاط الانقسامي للمجموعات المعالجه بالتاموكسيفين. يستنتج من البحث أن التغيرات في الكروموسومات نتيجة معالجة الأمهات بالتاموكسيفين يمكن أن يكون لها تأثير علي الأجنه بما يؤدي الي تشوهها أو الاجهاض للامهات. و لذلك يجب اتخاذ الحذر عند المعالجه به أثناء فترة الحمل.