Global Journal of Pharmacology 8 (2): 181-188, 2014
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2014.8.2.82225

Effects of Raloxifene and Strontium Ranelate on the Chromosomes of Virgin and Pregnant Female Mice

Hanaa M. Roshdy and Salwa M. Kassem

Department of Cell Biology,
National Research Center, Dokki, Cairo, Egypt

Abstract: Two new drugs for osteoporosis were approved at the recent period-a new generation selective estrogen receptor modulator, raloxifene and a drug with a novel mechanism of action, strontium ranelate. The safe use of raloxifene and strontium ranelate in the females before and during pregnancy and their embryos has not been adequately studied. In order to evaluate the cytogenetic effects of the two drugs before and during pregnancy.Raloxifene and strontium ranelate were administrated orally to the female mice (virgin and pregnant) with doses of 0.02 and 0.7 mg/kg/day respectively for 15 consecutive days. These doses equal to the recommended doses for human. After one day from the last treatment, the females were killed and cytogenetic analysis were conducted. The frequencies of chromosomal aberrations were increased significantly in the females (virgin and pregnant) and in the embryos treated with raloxifene but these increases were highly significant in the pregnant females than virgin females. While, in the females treated with strontium ranelate there was a slight significant increase in the frequencies of chromosomal aberrations in the pregnant females and there embryos but there was no significant increase in the females treated with strontium ranelate before pregnancy. Our results indicate that raloxifene has a mutagenic effects on both Virgin and pregnant females and embryos, while strontium ranelate has a slight mutagenic effects on the pregnant females and their embryos but does not have a mutagenic effect on the virgin females.

Key words: Osteoporosis · Raloxifene · Strontium Ranelate · Female Mice · Embryos · Chromosomal Aberrations

INTRODUCTION

Osteoporosis is one of the major health problems especially for women. The world "osteoporosis" Literally means porous bones. It is a bone disorder characterized by decreased bone strength as a result of reduced bone quantity and quality, the frequency of osteoporosis is constantly increasing all over the world. This pathology generates several problems, mostly due to fragility fractures, the worst consequence of impaired bone quality. Osteoporotic fractures often cause disability and loss of independence, significant pain and deformity. If fracture union is not achieved, the patient may suffer from long-term disability [1].

Many diseases associated with osteoporosis as Endocrine disorders like hypothyroidism hyperthyroidism, diabetes mellitus type I and type II. Pregnancy and lactation may cause reversible bone loss. Rheumatoid arthritis. Renal insufficiency which the bone formation is not regular and the bones so formed are porous and spongy and hematological disorders like, leukemia and lymphoma [2].

Several classes of drugs are used in the preventation of osteoporosis; many animal studies have demonstrated that the drugs commonly used against osteoporosis can positively influence fracture repair and implant Osseo integration. Two new drugs for osteoporosis were featured at the recent international osteoporosis foundation (IOF) [3] as a new-generation selective estrogen receptor modulator (Raloxifene) and a drug with a novel mechanism of action (Strontium ranelate). Raloxifene is a non steroidal estrogen-receptor modulator (SERM) which is used for prevention and treatment of osteoporosis. One of the consequences of the women's health initiative has been increased interest in (SERMs), because of the potential to retain the most of the

Corresponding Author: Hanaa M. Roshdy, Department of Cell Biology, National Research Center Dokki, Cairo, Egypt.

beneficial effect of estrogen while avoiding some of the adverse effects. Raloxifene appears to reduce the risk of breast cancer and decreases the serum level of lipoprotein and cholesterol.

As with estrogen, raloxifene effect occur when the drug binds tightly to estrogen receptors, which alters gene transcription in susceptible cells. Raloxifene bone effect appear to be mediated through osteoblasts, which result in the promotion of bone deposition and reduce of bone resorption that restores the balance between bone formation and bone resorption [4].

Strontium ranelate is a new orally administrated agent recently approved in Europe for the treatment of osteoporosis to reduce the risk of vertebral and hip fractures. As an alkaline earth element, strontium has close similarities with calcium in its absorption in the gut, in corporation into bone and relatively high renal tubular re-absorption in the kidneys. Since strontium is naturally present in trace amounts in the human body, treatment with strontium ranelate is simply making more stable strontium available for incorporation into bone.

Strontium ranelate has mechanism of action appears to be different from the other treatment firstly as it works by reducing bone resorption and stimulating bone formation leading to re-balancing of bone turnover in favour of bone formation.

Strontium ranelate stimulates the calcium sensing receptors and leads to the differenation of pre-osteoblast to osteoblast which increases the bone formation. Strontium ranelate also stimulates osteoblasts to secrete osteoprotegerin in inhibiting osteoblasts, formed from pre-osteoclasts, which leads to the decrease of bone resorption. Also, the drug is effective for preventing vertebral fractures not only in young osteoporotic women but also in the elderly patients [5].

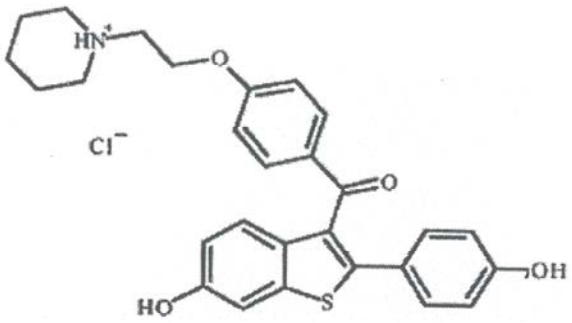
All data considered make raloxifene and strontium Ranelate a first-choice treatment in the preventation of osteoporosis.

At present no adequate data is available that illustrates the safety use of raloxifene and strontium ranelate in the females before and during pregnancy. Therefore, the present study was undertaken to determine the cytogenetic and mutagenic effects of raloxifene and strontium ranelate when they given orally for 15 consecutive days before pregnancy and during pregnancy and we examined also their effects on the embryos and compared their effects with the controls.

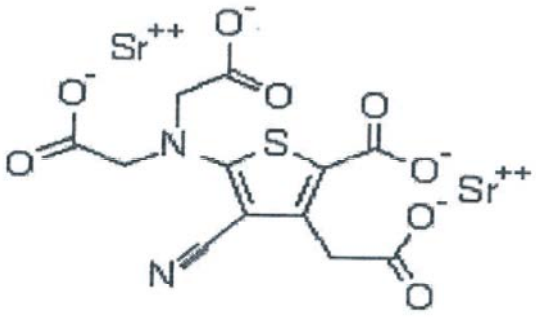
MATERIALS AND METHODS

Drugs:

- Evista (Raloxifene hydrochloride) was obtained from (Pharopharma- Cairo-Egypt). The chemical structure is the chemical name is methanone, [6-hydroxy-2-4-hydroxyphenyl] benzo[b]thien-3-l]-[4-[2-(1-piperidinyl)ethoxy]phenyl]-hydrochloride. Raloxifene hydrochloride has the empirical formula $C_{28}H_{27}NO_4S$-HCl. Raloxifene HCl is an off-white to-pale a yellow solid and is very slightly soluble in water. Evista is supplied in a tablet for oral administration each tablet contains 60 mg of raloxifene HCl. The recommended dose is 60 mg/day for human.



- Strontium ranelate was obtained from (Servier), astrontium (II) salt of ranelic acid is a medication for osteoporosis marked as (Protos). The chemical name is distrontium renelate, 5-(Carboxymethyl) amino]-2-carboxy-4-cyano-3-thiopheneacetic acid. The molecular structure is strontium ranelate has a molecular formula $C_{12}H_6N_2O_6SSr_2$ and a molecular weight 513.50. It is freely soluble in water. The recommended dose of protos (strontium ranelate) is 2g/day for human.



MATERIALS AND METHODS

Virgin female mice weighting 25-30 gm were acquired in pathogen free, well ventilated room in order to enable the animals to acclimatize to their environment. Drinking water and food supplied add libitum. Females were divided into two groups the first group were used for studding the effect of the treatments in virgin females and the second group were used for studding the effect of the treatments in pregnant females as following:

The First Group Were Divided into Three Subgroups: The first part of five virgin females were administrated orally with a single dose of 0.02 mg/kg/day Raloxifene HCl. This dose equal to the recommended dose for human after modified to suit the small weight of albino mice according to Pagat and Barnes [6].

The second part of five virgin females were administrated orally with a single dose of 0.7 mg/kg/day strontium ranelate. This dose equal to the recommended dose for human after modified to suit the small weight of albino mice according to Pagat and Barnes [6].

The third part of five virgin females served as control were administrated orally with distilled water.

The second group: Females were housed with adult males by ratio of 3:1 after one day of mating the females which exhibiting a vaginal plug were considered as pregnant. The day of the appearance of vaginal plug was considered as the first day of pregnancy.

The pregnant females were weighted and caged individually and divided into three subgroups:

The first subgroups of five pregnant females were administrated orally from the day 3 to the day 18 of pregnancy with a single dose of raloxifene HCl 0.02 mg/kg/day.

The second subgroups of five pregnant females were administrated orally from day 3 to day 18 of pregnancy with a single dose of strontium ranelate 0.7 mg/kg/day.

The third subgroups of five pregnant females served as control were administrated orally with distilled water.

After 15 days of treatments with raloxifene HCl and strontium ranelate the virgin and pregnant females were killed, the bone marrow of females randomly selected from each part of pregnant and virgin females to study the chromosomal abnormalities.

Chromosomes Preparation from Bone Marrow Cells of Virgin and Pregnant Females: Chromosomes from bone marrow cells were prepared according to methods of HUS and Patton [7] and Yosida *et al*. [8]. Bone marrow cells were collected in T.C.M. 199 culture media and colchicines was added to the tube (2ml of 0.05 colchicine) then, the cells were incubated at 37°C for 90 min. After centrifugation 5ml of hypotonic solution of 0.56% KCl was added and the pellet suspended and incubated at 37°C for 30 minutes. After centrifugation the cells were fixed in freshly prepared 3:1 methyl alcohol-glacial acetic acid then two or three drops of cell suspension were dropped on a clean slide, the slides were stained in 10% with Giemsa stain for 25 minutes.

Chromosomes Preparation from Embryonic Cells: Chromosomes preparations from embryonic cells were prepared according to Romagnano *et al*. [9]. Embryos livers were collected from each group and placed in 5ml T.C.M. 199 media, 2mL of 0.05 colchicine was added for each tube and incubated at 37°C for 90 minutes, then an amount of 5mL of hypotonic solution of 0.56% KCl was added to the pellet and the cells were incubated at 37°C for 20minutes, 5ml of fresh fixative (3 methyl alcohol: 1 glacial acetic acid) were added to the cells. After that two or three drops from the cell suspension were added to the surface of clean slides, air-dried, stained with 5% Giemsa stain and examined for chromosomal aberrations.

50 metaphase spreads were examined for each female and embryo, scoring the different types of chromosomal aberrations (structural and numerical).

Statistical analysis: The data of chromosomal aberrations in the females and embryos were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran[10]. Least significant differences were used compare between means according to Waller and Duncan [11] at probability 5%.

RESULTS

Chromosomal Aberrations

In the Virgin Females: Means ± S.D. values of chromosomal abnormalities are given in Table (1).

In the group of virgin females treated with strontium ranelate for 15 consecutive days. It can be seen that the frequencies of the all types of structural and numerical aberrations were in the same limit of control group, there

Table 1: The effect of oral administration of strontium ranelate and raloxifene in the virgin females.

	Structural aberrations								Numerical aberrations			
Treatment	Chromatid gap	Chromatid break	Deletion	Fragment	Centro- meric	Endo-metosis	ring	T.S.A.	<40	>40	polyploidy	T.N.A.
Control	3.67^{a}±0.577	1.67^{a}±0.577	2.00^{a}±0.00	3.00^{a}±0.00	3.00^{a}±0.00	3.67^{a}±0.577	0.00^{a}±0.00	16.67^{a}±1.55	3.33^{a}±0.577	2.33^{a}±0.577	0.33^{a}±0.577	6.00^{a}±1.00
Strontium ranelate	4.00^{a}±0.00	2.00^{a}±1.00	1.67^{a}±0.577	2.67^{a}±0.577	2.67^{a}±0.577	4.00^{a}±0.00	0.67^{ab}±0.577	17.67^{a}±1.155	3.67^{a}±0.577	0.67^{ab}±0.577	0.67^{AB}±0.577	7.00^{a}±0.00
Evista Raloxifene	6.00^{b}±1.000	4.33^{b}±0.577	4.00^{b}±1.00	4.00^{a}±1.00	5.67±0.577	6.67^{b}±0.577	1.33^{b}±0.577	32.00^{b}±1.00	6.00^{b}±1.00	1.67^{b}±0.566	1.67^{b}±0.566	12.33^{b}±0.577

Means of different letters (a,b,c,d) in the same column are significantly different.
The column with the same letters is not significant. 50 metaphase were examined from each animals.

Table 2: The effect of oral administration of strontium ranelate and raloxifene in the pregnant females

	Structural aberrations								Numerical aberrations			
Treatment	Chromatid gap	Chromatid break	Deletion	Fragment	Centro- meric	Endo-metosis	Ring	T.S.A.	<40	>40	Polyploidy	T.N.A.
Control	3.00^{a}±0.00	2.00^{a}±0.00	1.33^{a}±0.577	2.00^{a}±0.00	2.33^{a}±0.577	3.67^{a}±0.577	0.00^{a}±0.00	14.33^{a}±0.577	4.67^{a}±0.577	2.33^{a}±0.577	0.33^{a}±0.577	7.33^{a}±0.577
Strontium ranelate	4.00^{a}±1.00	2.67^{a}±0.577	1.67^{a}±0.577	2.33^{a}±0.577	3.33^{a}±0.577	4.00^{a}±1.00	0.67^{a}±0.577	18.67^{b}±0.577	4.67^{a}±0.577	3.33^{a}±0.577	1.00^{b}±0.00	9.67^{b}±0.577
Evista Raloxifene	6.67^{b}±0.577	4.67^{b}±0.577	4.67^{b}±0.577	5.33^{b}±0.577	5.33^{b}±0.577	6.00^{b}±1.00	2.33^{b}±0.577	34.67^{c}±0.577	6.33^{b}±0.577	5.67^{b}±0.577	2.00^{c}±0.00	14.00^{c}±1.00

Means of different letters (a,b,c,d) in the same column are significantly different.
The column with the same letters is not significant.50 metaphase were examined from each animals.

Table 3: The effect of oral administration of strontium ranelate and raloxifene on embryos

	Structural aberrations								Numerical aberrations			
Treatment	Chromatid gap	Chromatid break	Deletion	Fragment	Centro- meric	Endo-metosis	Ring	T.S.A.	<40	>40	polyploidy	T.N.A.
Control	2.33^{a}±0.577	2.00^{a}±0.00	1.33^{a}±0.577	2.00^{a}±1.00	2.00^{a}±0.00	2.33^{a}±0.577	0.00^{a}±0.00	12.00^{a}±1.00	2.00^{a}±0.00	1.67^{a}±0.577	0.00^{ac}±0.00	3.67^{a}±0.577
Strontium ranelate	3.00^{ab}±0.00	2.33^{a}±0.577	1.67^{a}±0.577	2.67^{ab}±0.577	3.00^{b}±0.00	2.33^{a}±0.577	0.67^{a}±0.577	15.67^{b}±0.577	2.67^{a}±0.577	2.33^{a}±0.577	1.00^{b}±0.00	6.00^{b}±1.00
Evista Raloxifene	4.33^{b}±1.155	4.00^{b}±1.00	3.33^{b}±0.577	4.00^{b}±1.00	4.67^{c}±0.577	3.33^{a}±0.577	1.33^{a}±1.155	26.67^{c}±0.577	4.67^{b}±0.577	3.67^{b}±0.577	1.67^{c}±0.77	10.00^{c}±1.000

Means of different letters (a,b,c,d) in the same column are significantly different.
The column with the same letters is not significant.50 metaphase were examined from each animals.

was no significant difference between strontium group and control group. The frequencies of the total structural and numerical aberration for strontium group were (17.67 and 7.00) compared with (16.67 and 6.00) for the control group.

While, in the group of virgin females treated with raloxifene there were a significant increases in the total number of structural and numerical ($P < 0.05$) aberrations compared with control. The frequencies of the total number of structural and numerical aberrations for raloxifene were (32 and 12.33) compared with (16.67 and 6) for control group.

In the Pregnant Females: Means ± S.D. values of chromosomal abnormalities are given in table (2). In the group of pregnant females treated with strontium ranelate from day 3 to day 18 of pregnancy, the frequencies of structural and numerical aberrations increased significantly than that of control group. The frequencies of the total structural and numerical aberrations for strontium ranelate group were (18.67 and 9.67) compared with (14.33 and 7.33) for control group.

Also, in the group of pregnant females treated with raloxifene the frequencies of the total structural and numerical aberrations were increased highly significantly from that of the control group.

The total structural and numerical aberrations in the group of pregnant females treated with raloxifene were (34.67 and 14) compared with (14.33 and 7.33) for the control group.

In the Embryos: Means ± S.D. values of chromosomal abnormalities are given in Table (3). Cytogenetic examination in embryos treated with strontium ranelate showed a slight significant increase in the total number of structural and numerical aberrations as compared with embryo control group. The frequencies of structural and numerical aberrations for strontium embryos group were (15.67 and 6.00) compared with that of control (12.00 and 3.67) respectively.

On the other hand, cytogenetic examination in the embryo group treated with raloxifene showed a highly significant increases in the total number of structural and numerical aberrations compared with the control group.

Table 4: Comparison between Control groups in the virgin females and pregnant females

	Structural aberrations								Numerical aberrations			
Treatment	Chromatid gap	Chromatid break	Deletion	Fragment	Centro meric	Endo-metosis	Ring	T.S.A.	<40	>40	Polyploidy	T.N.A.
Control before pregnancy	3.67^{a}±0.577	1.67^{a}±0.577	2.00^{a}±0.00	2.67^{a}±0.577	3.00^{a}±0.00	3.67^{a}±0.577	0.00^{a}±0.00	16.67^{a}±1.155	3.33^{a}±0.577	2.33^{a}±0.577	0.33^{a}±0.577	6.00^{a}±1.00
Control during pregnancy	3.00^{a}±10.00	2.00^{a}±0.00	1.33^{a}±0.577	2.00^{a}±0.00	2.33^{a}±0.577	3.67^{a}±0.577	0.00a±0.00	14.33^{b}±0.577	4.67^{b}±0.577	2.33^{a}±0.77	0.33^{a}±0.577	7.53^{a}±0.577

Means of different letters (a,b,c,d) in the same column are significantly different.
The column with the same letters is not significant.50 metaphase were examined from each animals.

Table 5: Comparison between the effect of strontium ranelate on virgin females and pregnant females

	Structural aberrations								Numerical aberrations			
Treatment	Chromatid gap	Chromatid break	Deletion	Fragment	Centro- meric	Endo-metosis	Ring	T.S.A.	<40	>40	Polyploidy	T.N.A.
Virgin females	4.00^{a}±0.00	2.00^{a}±1.00	1.67^{a}±0.577	2.67^{a}±0.577	2.67^{a}±0.577	4.00^{a}±0.00	0.67^{a}±0.57	17.67^{a}±1.755	3.67^{a}±1.755	2.67^{a}±0.577	0.67^{a}±0.577	7.00^{a}±0.00
Pregnant females	4.00a±1.00	2.67^{a}±0.577	1.67^{a}±0.577	2.33^{a}±0.577	3.33^{a}±0.577	4.00^{a}±0.00	0.67^{a}±0.577	18.67^{a}±0.577	18.67^{a}±0.577	3.67^{a}±0.577	1.33^{a}±0.577	9.67^{b}±0.577

Means of different letters (a,b,c,d) in the same column are significantly different.
The column with the same letters is not significant.50 metaphase were examined from each animals.

Table 6: Comparison between the effect of raloxifene in virgin females and pregnant females

	Structural aberrations								Numerical aberrations			
Treatment	Chromatid gap	Chromatid break	Deletion	Fragment	Centro- meric	Endo-metosis	Ring	T.S.A.	<40	>40	Polyploidy	T.N.A.
Virgin females	6.00^{a}±1.00	4.33^{a}±0.577	4.00^{a}±1.00	4.00^{a}±1.00	5.67^{a}±0.577	6.67^{a}±0.177	1.33^{a}±0.577	32.00^{a}±1.000	6.00^{a}±1.00	4.67^{a}±0.577	1.67^{a}±0.577	12.33^{a}±0.577
Pregnant females	6.67^{a}±0.577	4.67^{a}±0.77	4.67^{a}±0.577	5.33^{a}±0.577	5.33^{a}±0.577	2.33^{a}±0.577	2.33^{a}±0.577	34.67^{b}±0.577	6.33^{a}0.577	5.67^{a}±0.577	2.00^{a}±0.00	14.00^{b}±1.00

Means of different letters (a,b,c,d) in the same column are significantly different.
The column with the same letters is not significant.50 metaphase were examined from each animals.

The frequencies of structural and numerical aberrations for raloxifene embryo group were (25.67 and 10) compared with that of control (12 and 3.67) respectively.

Comparison Between All Groups (Control and Treated) Before and During Pregnancy:

Comparison Between Control Groups on the Females Before and During Pregnancy: Means ± S.D. and results are given in Table (4). There were no significant differences between all the types of structural and numerical aberrations in the two control groups of the virgin and pregnant females.

Comparison Between the Effect of Strontium Ranelate Treatments on the Females Before and During Pregnancy: Means ± S.D. and results are given in Table (5) cytogenetic examination showed that there were no significant differences between all types of structural and numerical aberrations in the two treated groups of strontium ranelate but on the other hand there was a significant increase in the total number of structural and numerical aberrations in the females treated with strontium during pregnancy than that treated with strontium before pregnancy. This means that pregnant females were more affected by strontium ranelate than virgin females.

Comparison Between the Effect of Raloxifene Treatments on the Females Before and During Pregnancy: Means ± S.D. and results are given in table (6) when comparing the frequencies of the total structural and numerical aberrations between the virgin females and the pregnant females treated with raloxifene we found that pregnant females had more frequent in the chromosomal aberrations than those of virgin females. This means that the effect of raloxifene is more frequent during pregnancy than before pregnancy.

DISCUSSION

Osteoporosis is a silent condition where the bones become weak and prone to fracture. Bone is living tissue that is in a constant state or regeneration. That is, the body removes old bone (called bone resorption) and replaces it with new bone (bone formation). By their mid-30, most people begin to slowly lose more bone than can be replaced. As a result, bones thinner and weaker in

structure. The most common fractures occur at the spine, wrist and hip. The main goal of treating osteoporosis is to prevent such fractures in the first place. Many factors may be increased the risk of developing osteoporosis such as endocrine diseases, low calcium and vitamin D intake, age, genetic factors, low estrogen levels and pregnancy.

Pregnancy associated osteoporosis is believed to be a rare condition that is usually found in the third trimester of a woman's pregnancy or after giving birth. It is usually occurs during a woman's first pregnancy, is temporary and does not happen again. Women affected usually complain of back pain, have a loss of height and have vertebral fractures [12].

As of 1996, there had been 80 cases of this condition reported. Researchers do not know if this condition occurs as a result of pregnancy or because of other health problems the woman had.

Things that may cause this condition, such as genetic factors or steroid use, are being studied. Even through there is stress on a pregnant women's calcium supply and calcium leaves her body more often because of frequent urination, other changes during pregnancy, like increases in estrogen and weight gain, may actually help bone density. The goal of osteoporosis treatment is the preventation of bone fractures by stopping bone loss and by increasing bone density and strength. Osteoprosis treatment and preventation measures are consuming a balanced diet with adequate calcium and vitamin D and using medications that stop bone loss and increase a bone strength such as raloxifene (Evista) a new generation selective estrogen receptor modulator it has attracted attention, especially after the recent move a way from hormone replacement therapy and a drug with a noval mechanism of action, strontium ranelate (protos), as it works both by reducing bone resorption and stimulating bone formation [13].

In fact the safe use of the two drugs has not been adequately studied in females before and during pregnancy.

In our study, the administration of virgin females with strontium ranelate once daily for 15 days showed no significant increase in the chromosomal aberrations in the bone marrow cells compared with control group.

While, the administration of virgin females with raloxifene for 15 days caused a significant increase in the chromosomal aberrations of bone marrow cells compared with the control group these results is agreement with Judith *et al*. [14] who observed that the treatment with raloxifene in mice and in rats with a doses over the human dose caused an increased in the incidence of ovarian tumors in the both species.

Similar result was observed by Vogel *et al*. [15] who found that the treatment of female rodents with raloxifene throughout their lives produced a specific hormonal imbalance this imbalance are known to result in ovarian tumors in rodents, which have not been observed in women who have received, raloxifene.

Also, similar result was obtained by Cebesoy *et al*[16] who observed that in many animal studies strontium ranelate did not exhibit any toxic effects at a dose up to 625 mg/kg/day. While, negative results were obtained by Olaf *et al* [17] who found that the treatment with a repeated doses of strontium ranelate in rats and mice caused a cytotoxic effects in bone marrow cells of these animals.

Also, negative results were obtained by Mincey *et al* [18] who found that the treatment with raloxifene in animals and humans reduce the risk of cancer. In addition, cytogenetic and developmental toxicity in embryos may occur through a direct effect of some chemicals or hormones on the embryo, fetus or indirectly through toxicity of the drug to the mothers and the placenta, or most commonly as a combination of the two concepts. Maternal conditions are capable of adversely affecting the developing organism in the uterus Khera [19].

In our study, the administration of strontium ranelate with a dose equal to the recommended dose in human to the female mice during pregnancy caused a slight significant increase in the chromosomal aberrations in the pregnant females and their embryos. This result is agreement with Ahmet *et al*. [20] who found that when strontium ranelate administrated to the female rat during pregnancy strontium accumulated in the uterus and in the rate milk and caused toxic effect on the embryos. While these finding was in agreement with Tanriover *et al.* [21] who observed that after oral administration of strontium ranelate to the women during pregnancy no clastogenic or toxic effects on the pregnant women was observed.

Also, in the present study, we found that when pregnant female mice were administrated orally from day 3 to day/18 of pregnancy with a dose of raloxifene equal to the recommended dose in human raloxifene caused a highly significant increase in the total chromosomal aberrations in the bone marrow and embryonic cells.

These finding was agreement with David *et al.* [22] who found that in animal studies raloxifene caused foetal malformations in rabbits and abnormalities in the reproductive systems and impaired reproductive function in the female offspring of rats.

Also these finding was agreement with Judy *et al.* [23] who found that when raloxifene was administrated to the pregnant woman a development effects were observed at a dose equal to the recommended dose for human. Positive result was observed by Richard and Paul [24] who reported that when raloxifene administrated by oral gavages to mated female rats during the preimplantation period at 0.1 to 10 mg/kg/day raloxifene delayed and disrupted embryo implantation and reduced litter size.

CONCLUSION

In conclusion our results indicated that raloxifene (Evista) had a significant mutagenic and cytotoxic effects on females (virgin and pregnant) and on embryos. This may be as a result that raloxifene belongs to the class of selective estrogen modulators. Its action are mediated through high affinity binding to estrogen receptors and regulation of gene expression. Treatment of females (virgin and pregnant) with raloxifene throughout their lives produced specific hormonal imbalances. Such imbalances are known to result ovarian and uterus tumors.

Also, in the present study we found that the treatment of female mice with (strontium ranelate) before pregnancy had no mutagenic or cytotoxic effects to the virgin female mice. However, the treatment with strontium ranelate during pregnancy caused a slight significant increase in the chromosomal aberrations of pregnant females and embryos. This may be as a result that strontium ranelate has the same structure of calcium and can enter the bone without any side effect, however during pregnancy strontium can passes into the female uterus and cause a slight toxicity to the pregnant female and embryos.

REFERENCES

1. Tarantino, U., G. Garnata, I. Cerocchi, D. Lecce, R. Lundusi and M. Celi., 2007. Surgical approach to fragility fractures: problems and perspectives. Aging Clin. Exp. Res., 19: 12-21.
2. Cumming, S.R. and J. Melton, 2002. Epidemiology and Outcomes of osteoporosis fractures. Lancet, 35: 1761-7.
3. Rio, B., 2004. new drugs for osteoporosis: Lasofoxifene and strontium ranelate. Osteoporos Int, 15: 19-22.
4. Bolognese, M.A., S.R. Weiss and M.P. Ettinger, 2004. Raloxifene: anext generation selective estrogen receptor modulator (SERM) for the presentation of bone loss in postmenopausal women. Osteoporos Int, 15: 19-23.
5. Morie, P.J., 2005. Strontium ranelate a noval mode of action of optimizing bone formation and resorption. Osteoporos Int, 16: 7-10.
6. Pagat and Barnes, 1964. Evaluation of drug activities, No. 1. Academic Press.
7. Hus, T.I.C. and J.L. Patton, 1969. Bone marrow preparations for chromosome studies, In bernischek comparative mammation cytogenetics spring erverlag, pp: 454-460.
8. Yosida, T.H., K. Truchiya and K. Moiwaki, 1971. Frequency of chromosome polymorphism of Rattus rattus collected in Japan. Chromosoma, 30: 33.
9. Romagnano, A., C.L. Richer and M.A. Perrove, 1985. A direct technique for the preparation of chromosomes from embryo equine embryos. Can. J.Genet Cytol, 27: 365-369.
10. Snedecor, G.W. and W.G. Cochran, 1990. Statistical methods 9th ed Lowa State Univ. Press, Lova, U.S.A.
11. Waller, A. and D.B. Duncan, 1969. Multiple range and multiple test. Bionetries, 11: 1-24.
12. Davey, M.R., J.T. Villiers, S. Lipschitz and J.M. Pettifer, 2012. Pregnancy and lactation associated osteoporosis. Journal of Endocrinology, Metabolism and Diabetes of South Africa, 17: 3-10.
13. Riggs, B.L. and L.C. Hartmann, 2003. Selective estorgene-receptor modulators-Mechanisms of action and application to clinical practice. N Engl J Med, 13: 618-629.
14. Judith, H., F. Lawrence, B. Judy and F. Paul, 1998. The selective estrogen modulator raloxifene: reproductive assessments following exposure in female rats. Reproductive toxicology, 12: 233-245.
15. Vogel, V.G., J.P. castantino and D.L. Wickerham, 2006. Effects of tamoxifene VS raloxifene on the risk of developing in vasive breast Cancer and other disease outcomes. Jama, 295: 1-15.
16. Cebesoy, O., E. Tutar, K.C. Kose, Y. Battaa and C. Bagci, 2007. Effect of strontiu m ranelate on fracture healing in rat tibia. Joint Bone Spine, 74: 590-593.
17. Olaf, B., K. Niels and N. Stigpors, 2009. Effects of long-term treatment with strontium ranelate on bone strontium content. Bone, 45: 200-206.

18. Mincey, B.A., T.J. Moraghan and E.A. Perez, 2000. Preventation and treatment of osteoporosis in women with breast cancer. Mayo Clinc Proc, 75: 827-829.
19. Khera, K.S., 1984. Maternal toxicity: a possible factor in embryo-fetal deaths and fetal malformations of rodent-rabbit. Teratology, 31: 129-153.
20. Ahmet, N.S., G. Tokman, G. Durmus G.E. Kukln and M. Kucuk, 2009. Effects of strontium ranelate, raloxifene and misoprostol on bone mineral density of ovariectomized rats. Eur Jobstel Gynecol reprod Biol, 147: 192-194.
21. Tanriover, M.D., S.G. Oz, T. Sozen, A. Kilicarslan and G.S. Guyen, 2009. Pregnancy and Lactation-associated osteoporosis with severe vertebral deformities: can strontium ranelate be a new alternative for the treatment. Spine. J, 9: 20-4.
22. David, C., G. Kelly, S. Judy and F. Paul, 1998. The selective estrogen receptor modulator, raloxifene: reproductive assessments following pre implantation exposure in mated female rats. Reproductive toxicology, 12: 247-259.
23. Judy, S., B. Henry and F. Paul, 1998. The selective estrogen receptor modulator, raloxifene: an overview of non clinical pharmacology and reproductive and developmental testing. Reproductive toxicology, 12: 217-221.
24. Richard, B. and F. Paul, 1998. The selective estrogen receptor modulator, raloxifene: segment II studies in rats and rabbits. Reproductive Toxicology, 12: 261-270.