Cytotoxicity impacts of Omnipaque 350 and X-ray on mice chromosomales

Muthana Ibrahim Maleek . Ayat Ali Mahmud AL-jobory
Department of Biology, College of Science
University of Wasit, Wasit Province, AL- Kut, Iraq

التأثيرات السمية لمادة ألاومنيباك350 والأشعة السينية على كروموسومات الفئران المختبرية

مثنى إبراهيم ملك و آيات علي محمود الجبوري

قسم علوم الحياة / كلية العلوم / جامعة واسط – محافظة واسط – الكوت

المستخلص

في هذه التجربة حسبت النسبة المئوية للتشوهات الكروموسومية وتكوين النويات الصغيرة و معامل الانقسام الخلوي في خلايا نخاع العظم للفئران المختبرية كطريقة لقياس قدرة الاومنيباك 350 على زيادة التشوهات الكروموسومية وتكوين النويات وتقليل معدل الانقسام بدون ومع الأشعة السينية. وقد استخدمت الفئران المختبرية و قسمت الى مجموعتين : مجموعة سيطرة ومجموعة معالجة. مجموعة السيطرة تضمنت 3 مجموعات أحداهما سيطرة سالبة عوملت ب (0,25 مل ماء مقطر) والأخرى شععت بالأشعة السينية (ثلاث جرعات متوالية من 60 KeV) والمجموعة الثالثة عوملت (0,25مل من ألاومنيباك) لمدة 24 ساعة حقنت داخل الغشاء البريتونى. أما مجاميع المعالجة فقد عوملت بمادة الاومنيباك والأشعة معا و لكن في أوقات مختلفة من خلال حقن مادة الاومنيباك قبل او بعد او بين تشعيعين. بعد تحليل بيانات الاختبار إحصائيا, بينت النتائج أن مادة الاومنيباك 350 وحدها أدت الى زيادة معنوية في التشوهات الكروموسومية وتكون النويات الصغيرة مع انخفاض في معدل الانقسام($p<0.05$) وتزداد هذه النسبة بتزامن الاومنيباك مع الإشعاع.

الكلمات المفتاحية :ألاومنيباك 350 , الأشعة السينية , السمية الوراثية, التشوهات الكروموسومية , معدل انقسام الخلايا و النوى الصغيرة.

Abstract

In this paper, the percentage of chromosomal aberrations, micronuclei and mitotic index were measured in mice bone marrow as a method for assessing the ability of Omnipaque 350 to increase chromosomal aberrations, micronuclei and reduce mitotic index with or without X-ray. Laboratory mice were used for this purpose and divided into two groups: control groups and treated groups. Control groups consist of three groups, two are negative groups (the first received 0.25 ml of distilled water and the second was irradiated by three doses of 60 KeV) while the last one is positive group which was treated with 0.25ml of Omnipaque only for 24 hour. The treated groups are treated with Omnipaque and X-ray together but in different times by injection Omnipaque before, after and between two radiations. Experimental data was analyzed. The results demonstrated that Omnipaque alone has the ability to increase chromosomal aberrations, micronucleus and reduce mitotic index significantly ($P<0.05$).

Key words: Omnipaque 350, X-ray, Genotoxicity, Chromosomal aberrations, Mitotic index and Micronuclei.

Introduction

Contrast media (CM) are widely used in medical imaging for example computed tomography (CT scan), magnetic resonance images (MRI) and X-ray applications. Contrast medium is useful in distinguishing between normal and abnormal areas and it can be classified as; iodinated contrast media (ICM) and non-iodinated contrast media. Iodinated CM also can be divided into ionic and non-ionic (1). Structurally, a one benzene ring or two benzene rings may exist in CM forming monomer or dimer respectively (2). Omnipaque 350 (OP 350) or Iohexol (350 mg I/ml) is radiographic constant medium. The molecular formula of OP 350 is $C_{19}H_{26}I_3N_3O_9$. Iohexol has molecular weight of 821.14 (iodine content 46.36%) (3).Omnipaque 350 is non-ionic monomer iodinated CM that can be used for many diagnostic procedures (4, 5). X-ray is ionizing radiation has enough energy to ionize atoms or molecules in biological systems. X-ray was used in diagnostic radiology and capable to induce both gene mutations and chromosomal aberrations. X-ray directly damages DNA molecule or indirectly by forming reactive compounds that react with the critical molecules of cell such as DNA (6).There is a relationship between a contrast medium and X-ray.

Contrast medium works to block X-ray transition and improve images (7). In diagnostic radiology, iodinated contrast media are largely utilized. Previous study has demonstrated by cytogenetic analysis the effects of both X-ray and CM experimentally on cell culture *in vitro* (8). There are many results demonstrated the genotoxicity of CM in the lymphocyte cells of persons undergoing angiography. Many studies showed that many CM alone has the capacity to stimulate genotoxic impacts, and when combined with X-ray, genetic damage will increase. The radiological contrast media propose that CM increase the absorbed dose and may improve the blood cell sensitivity to the radiation induced cell damage (9, 10). The osmolality, viscosity, density and the number of iodine per milliliter in the solution are the most consequential properties of CM and related to their efficacy and safety. Therefore, CM shows chemotoxic effects due to their direct toxicity and physiological properties (11). This study was purposed to evaluate the cytogenetic effects of OP 350 on mice bone marrow stem cells when was given alone or combined within X-ray.

Materials and Methods

Dose and concentration of Omnipaque (OP) 350

Omnipaque (350mg I/ml) is the product of (GE Healthcare, Ireland). It was obtained from Al-Zahra Teaching Hospital. Each 1ml of OP contains 350 mg of Iodine. Our dose of OP 350 in this experiment is about 0.25 ml. This dose of OP 350 (0.25 ml) contains 90 mg of Iodine. The administration route of OP through the intraperitoneal injection .It was absorbed by organ tissue via continued accumulation (12).

Dose of X-ray

Mice were irradiated by ionizing radiation (X-ray) in AL-Zahra Teaching Hospital, Department of X-ray. CT scan is a medical diagnostic tool that can give multi doses (3 dimensional, 3D) of X-ray respectively. The dose of X-ray that was used in our experiment by X-ray machine is multi-doses of 60 KeV (3 doses of 60 KeV, mimicking the CT-scan effects) (12, 13).The animals were put in a plastic cage under the source of energy and made them recieved 3 doses of 60 KeV recpictively.

Laboratory animals

Sixty albino Swiss male mice were obtained from National Center for Drug Control and research / Ministry of Health / Baghdad. Their age ranged between (8-12) weeks and weighting (25 ±2) gm. They were divided into 6 groups, each group was put in a separated plastic cage under laboratory conditions.

Administration of laboratory animals

The animals were allocated into two main groups: control and treated groups.

Control groups

The animals (mice) of control groups were divided into three groups:-

- **Negative control group 1:** The animals were treated only with (0.25 ml) of distilled water. Negative control group 1 (10 animals) were killed after 24 hour. The mouse bone marrow samples were taken for cytogenetic analysis (MI, CA, and MN).
- **Negative control group 2:** The animals were exposured to 60 KeV for three doses of X- ray. Negative control group 2 (10 animals) were killed after 24 hr.
- **Positive control group:** The animals were treated with (0.25 ml) OP 350. Positive control group (10 animals) also was killed after 24 hr.

Treated groups

The mice in these groups were injected intraperitoneally with single dose of OP 350 (0.25ml) and irradiated by X-ray (3 doses of 60 KeV) in different times.

Three groups (10 mice for every group) were used for this experiment; these groups were divided as follow:

- **Group I**: The animals were injected firstly with OP 350 (0.25ml) then immediately irradiated by 60 KeV, 3 doses per animal.
- **Group II:** The animals were irradiated by X-ray (also 60 KeV, 3 doses per animal) then injected with OP 350 (0.25 ml).
- **Group III:** The animals were irradiated by 3 doses of X-ray (60 KeV) then injected with OP350 (0.25ml)and rradiated by 3 doses of X-ray again.

After 24hr. of last completion of each experiment, the mice were killed, samples of bone marrow were taken and cytogenetic analyses were carried out as described later.

Cytogenetic experiments

Chromosome preparation from somatic cells of the mouse bone marrow

The experiment was done according to Allen *et al* (14). Colchicine was injected 2 hr. before sacrifice. Mice were sacrificed by cervical dislocation. It was dissected and both of femur bones were excised. Bone marrow was aspirated by flushing with 5ml of phosphate buffer saline (PBS) in the centrifuge tube. The suspension was flushed in the tube properly to get good cell suspension and centrifuged for 10 min at 2000 rpm. Supernatant was discarded and the pellet was treated with pre-warmed (37°C) 5 ml of KCl and shaken well. Suspension incubated in a water bath at 37°C for 20 min. Pellet was treated with freshly 5ml prepared fixative solution (Methanol: Glacial Acetic Acid, 3:1) and shaken well then centrifuged for 10 min at 2000 rpm. Fixative was repeated 3 times to get debris free white pellet. Few drops from the tube were dropped vertically on the slide. Slides were kept overnight to dry then stained with (Giemsa's stain) and observed under microscope in 40 x and then in 100 x magnifications. A total of 100 well spread metaphase plates were scored for chromosomal aberrations (chromatid break, polyploidy, acentric fragment, ring and fragment) were counted and the data of scoring was expressed as percentage chromosomal aberrations.

Cytogenetic analysis

1-Mitotic index (MI) assay

The slides were examined under high power (40 X) of light microscope and (1000) of divided and non-divided cells were counted and the percentage rate was calculated for only the divided ones (metaphase cells) according to the following equation (15):-

$$\text{Metaphase Index (\%)} = \left(\frac{\text{Number of metaphase cells}}{\text{Total number of the cell(1000)}} \right) \text{x } 100$$

2-Chromosomal aberrations (CA) assay

The prepared slides were examined under the oil immersion lens (100X) of light microscope for 100 divided cells per each animal, and the cells had to be at the metaphase stage of the mitotic division where the chromosomal aberrations were clear and the percentage of these aberrations could be estimated (14).

3-Micronucleus MN assay

This experiment was done according to method of Schmid (16) as follow:-

The femur bone cleaned from tissues and muscles, and then gapped from the middle with a forceps in a vertical position over the edge of a test tube by a sterile syringe. One ml of human plasma (heat inactivated) was injected so as to wash and drop the bone marrow in the test tube. Then the test tubes were centrifuged at speed of 1000 rpm for 5 min. 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 hour. The slides were fixed with absolute methanol for 5 min., then stained with Giemsa stain for 15 min, then washed with distilled water and left to dry. Two slides for each animal were prepared for micronucleus test. The slides were examined under the oil immersion lens, and at least 1000 polychromatic erythrocytes (PCE) were examined for the presence of micronucleus. The micronucleus index was obtained using the following equation:

$$\text{MN Index} = \left(\frac{\text{Number of micronucle i}}{\text{Total count of PCE}} \right) \text{x } 100$$

Statistical analysis

The values of the investigated parameters were given in terms of mean ± standard error, and differences between means were assessed by analysis of variance (Two-sample T-test) using the computer program Minitab release (14.12) discovery Copyright 2004. The difference was considered significant when the probability value was less than $p < 0.05$.

Results and discussion

Table (1) shows the percentages of mitotic index in mice bone marrow for negative control group1in comparison with other groups (negative control group 2, positive control and treated groups). There is a

significant difference when we compared between negative control group 1(0.25 ml of D. W.) and other groups (negative control group 2, positive control and treated groups (I, II and III) and these differences may be back to the toxic effect of OP 350 alone or combined with X-ray by reducing the mitotic index (MI). All these results were significant at (P<0.05).

Table (1) : Percentages of mitotic index in mice bone marrow for negative control 1 and 2), positive control and treated groups

Groups	Mitotic index % *M±SE*
Negative control group 1 (0.25 ml of D.W)	**6.6 ± 0.3**
Negative control group 2 (three doses of 60 KeV X-ray)	**[a]*5.22 ± 0.086**
Positive control group (0.25 ml of OP 350 alone)	**[b]*4.00 ± 0.62**
Treated group 1 (OP350 +X-ray)	**[c]*3.140 ± 0.075**
Treated group 2 (X-ray + OP 350)	**[c]*3.320± 0.097**
Treated group 3 (X-ray+ OP 350 +X-ray)	**[c]*2.500± 0.12**

[a]Negative control group 2 Vs. Negative control group 1, [b] Positive group Vs. Negative control group 1, [C] treated group Vs. Negative group 1, *Significant at (P<0.05).

Table (2): Percentages of different types of chromosomal aberrations (CA) in mice bone marrow for negative control group (1 and 2) , positive control group and treated groups (*Mean* ± *SE*):-

Experimental Groups	Chromosomal aberrations %					
	Acentric Fragment %	Ring %	Polyploidy %	Break %	Fragment %	Total %
Negative control group 1 (0.25 ml of D.W.)	5.4 ± 1.75	0.000 ± 0.000	0.000 ± 0.000	0.2 ± 0.2	5 ± 0.707	10.6 ± 1.6
Negative control group 2 (3 doses of 60 KeV)	6.8 ± 2.18	0.6 ± 0.245	0.4 ± 0.245	0.8 ± 0.374	9.60 ± 1.29	[a*]18.2 ± 2.2
Positive control (0.25 ml of OP 350)	11.00 ± 2.55	4 ± 1.14	1.2 ± 0.583	2.4 ±1.94	18 ± 2.19	[b*]36.60 ± 4.3
Treated group I (OP 350 + X-ray)	25.8 ± 6.76	4.8 ± 1.32	1.2 ± 0.49	0.4 ± 0.245	20 ± 1.26	[c*]52.2 ± 8.1
Treated group II (X-ray + OP 350)	18.20 ± 5.00	2.4 ± 0.872	1 ± 0.447	0.2 ± 0.2	15.60 ± 2.91	[c*]37.40 ± 7.5
Treated group III (X-ray + OP 350 + X-ray)	35.60 ± 3.47	15.20 ± 3.71	1.8 ± 0.97	1.8 ± 0.347	25.60 ± 5.23	[c*]80.00 ± 6.7

[a] Negative control group 2 Vs. Negative control group 1, [b] Positive group Vs. Negative control group 1, [C] treated group Vs. Negative group 1, *Significant at (P<0.05)

Result of chromosomal aberrations (Table 2) in positive control group (0.25 ml of OP 350 only) showed a high frequency of total chromosomal aberrations (36.6 %) in mice bone marrow cells, this finding was significant (P<0.05) when compared with negative control group 1 (10.6%). While the result of total CAs of negative control group 2 (treated with three doses of 60 KeV X-ray) showed a low frequency of total chromosomal aberrations (18.2 %) in mice stem cells, but this result was also significant (P<0.05) when compared with significant (P<0.05) when compared with negative control group 1 (10.6%). Animals

in the three treated groups (I, II and III), which are treated with OP 350 and X-ray together but in different times (OP 350+X-ray =52.2%, X-ray+ OP 350 = 37.4%, X-ray +OP 350+X-ray = 80%) gave a high significant difference in mice bone marrow cells, when compared with negative control group 1 (10.6 %). But when the same dose of OP 350 was given between two irradiation, the result of total CAs showed a very high significant value (P<0.05) when compared with negative control group 1.

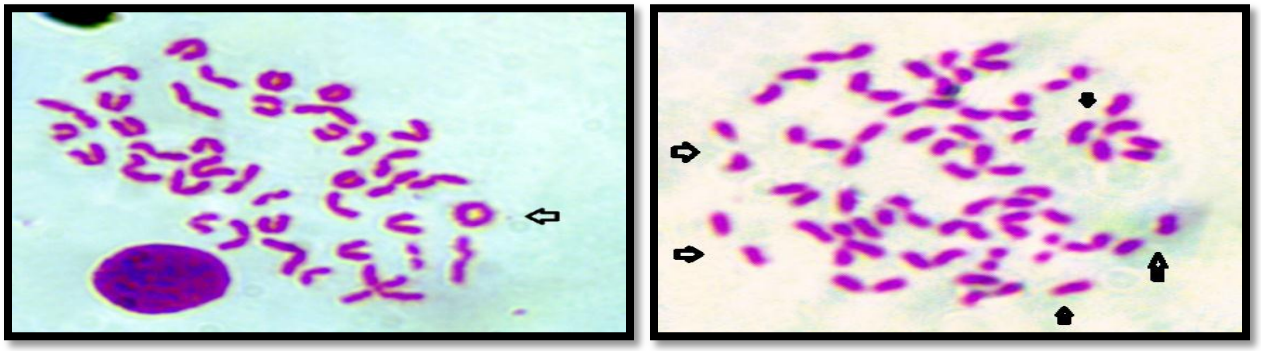
Table (3):- Percentages of micronuclei (MN) in bone marrow of mice for negative control group 1 and 2, positive control and treated groups (*Mean + SE*)

Groups	Micronucleus % *M*±SE
Negative control group 1(0.25 ml of D.W.)	**2.62 ± 0.12**
Negative control group 2 (3 doses of 60 KeV X-ray)	***[a]3.720 ± 0.23**
Positive control (0.25 ml of OP 350)	***[b]4.98 + 0.12**
Treated group 1 (OP 350 +X-ray)	***[c]5.54 + 0.29**
Treated group 2 (X-ray + OP 350)	***[c]5.060± 0.12**
Treated group 3 (X-ray+ OP 350 +X-ray)	***[c]8.780 + 0.45**

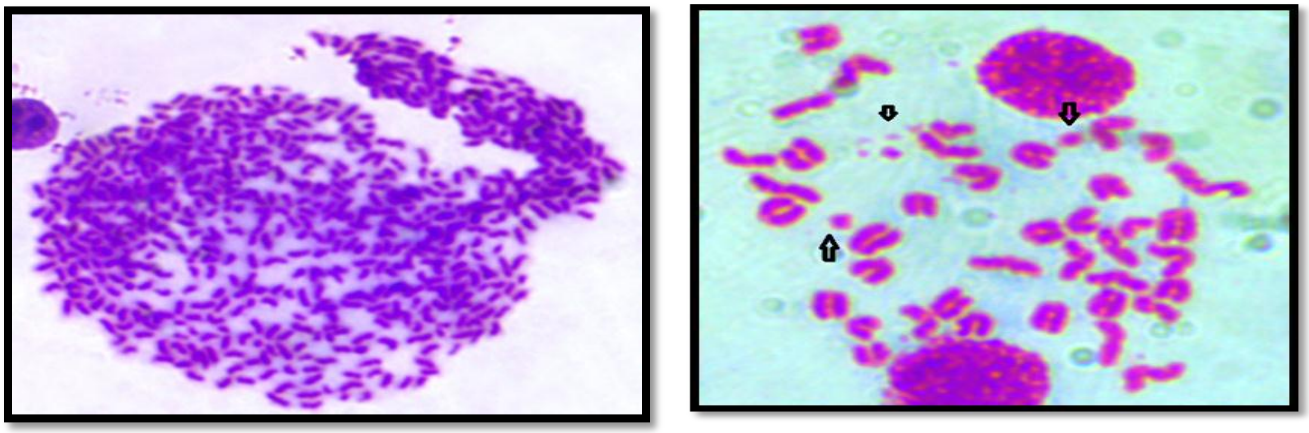
[a] Negative control group 2 Vs. Negative control group 1, [b] Positive group Vs. Negative control group 1, [C] treated group Vs. Negative group 1, *Significant at (P<0.05)

Table (3) shows the results of micronuclei (MN). The frequency of MN in negative control 1 appeared a significant differences when compared with positive group , negative control group 2 and all treated groups at (P<0.05).

The positive control showed a significant reduction in MI and a high increasing in CAs and MN. The reason of these results is related to the cytotoxic impact of OP 350 as OP 350 is iodinated CM contain iodine in its basic chemical structure, has cytogenic effects on MI by reducing cell division of mice bone marrow. Reducing of MI by OP 350 may be caused by antiproliferative properties of iodine atoms and their effects on arresting cell cycle (G1 and G2- M phases) (17). Iodine showed cytogenetic effects on MI by inhibition the activation of certain gene called Protein kinase B (PKB), also known as Akt, which is responsible for proliferation of cells (18). OP 350 is nonionic CM has triiodobenzoic acid. This triiodobenzoic acid is capable to reduce mitosis. Because the triiodobenzoic acid has



A B



C D

Figure (1):Shows different chromosomal aberrations in mice bone marrow (100X) injected only with OP 350. A: ring B: acentric fragment C: polyploidy D: fragment.

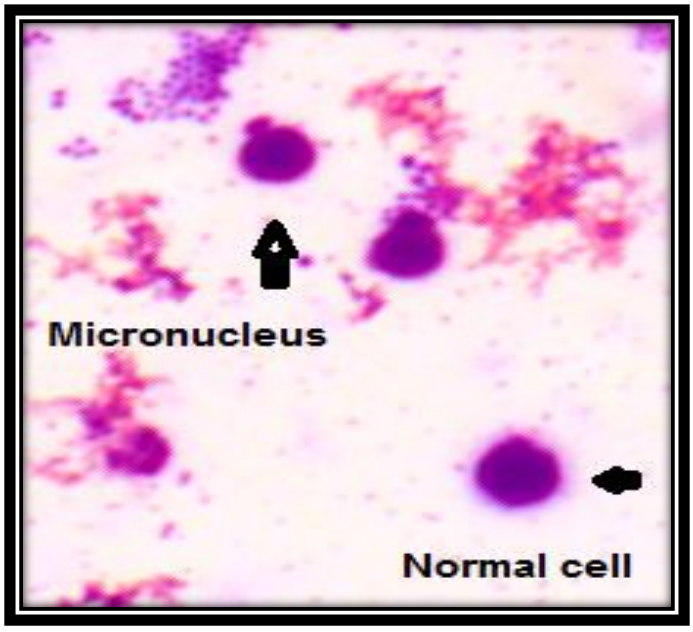


Figure (2): Formation of MN by OP 350 only (100X)

the capacity to produce chromosomal aberrations, the cell cycle will arrest and delay by the action of checkpoint (blocking of cell division) to prepare for mechanism of repair system (19).The ability of OP 350 (ICM) to form CAs and MN is related to one of the following reasons:- [1] Toxic iodine in triiodobenzoic acid has the capacity to react with nucleus and produce various protein and DNA fractions by iodinating double bonds.[2] Iodine can work as a strong free radical (20, 21).

Therefore, OP 350 can produce CA and MN in mice stem cells. Figure (1 and 2) showed different chromosomal aberrations (ring, acentric fragment, polyploidy, fragments) and MN in positive control group (OP 350 only).The results of negative control group 2 (X- ray treatment) demonstrated that X-ray can induce clastogenic effects on cells of mice bone marrow. One of these effects is reducing the normal cell division. X-ray can reduce MI significantly to (5.22% in comparison with negative control group1) ($P<0.05$) by delay cell transition from phase to another phase because the presence of CAs (DNA damage by action of X-ray) (22). The most common effect of ionizing irradiation is its capacity to react directly within the molecular components of cells or indirectly within water molecules leading to form water derived radicals. Free radical interacts with proximate molecules in a very stumpy time producing many fractions in chemical bonds or oxidation of the affected molecules. DNA is the main purporting for irradiation damaging as DNA perversions can die or mutate human or animal cells (23, 24). The results of the three treated groups

indicated the reducing of MI and increasing of CA and MN. These results are due to the interaction between the contrast media (OP 350) and X-ray effects. The interaction between OP 350 and X-ray was found. Omnipaque 350 is nonionic ICM has three atoms of iodine (which is toxic) attached within benzene ring at 2, 4, 6 sites, forming triiodobenzoic acid. Iodine atom itself can produce CAs and was used to prevent the biological tissues by attenuation of X-ray. When Omnipaque 350was injected, it would be absorbed rapidly by reticulo endothelial system (RES) and attached covalently to cell membrane. Therefore, when X-ray is absorbed by OP 350 (iodine), OP 350 within X-ray will hit DNA and produce many damages, loading to delay cell cycle and repair system. Antiproliferative properties of iodine atom and triiodobenzoic acid have many effects on cell cycle arresting (usually in G_1 phase and in addition to G_2/ M phase).They also can inhibit the activation of certain gene which is (Akt). On another hand, in exposure of X-ray, Iodine can attenuate X-ray to prevent the biological tissue. Emission of any type of radiation from radioiodine has the capacity to incapacitate chemical bonds everywhere of the cell, produce many fractions on DNA molecule and cause further cellular dysfunction (25, 26). Therefore, when radioiodine attenuates X-ray, it will emit this energy to the surrounding biological tissue, loading to produce more DNA damage. In our present study, decreasing of MI related to the antiproliferative properties of iodine. In addition, the dangerous interaction between ICM and X-ray may also inhibit the DNA repair system (9, 27 and 28). Some researchers proposed that there are varying between X-ray dose absorbed by blood cells of persons treating by angiocardiography in the presence or absence of CM. They determinate the effect of CM and X-ray dose using chromosomal abnormality assay and concluded two impacts of CM:- (a) Absorption of X-rays can be increased.(b) Contrast medium has the ability to form chromosomal fractions even in the absenteeism of X–rays (29).

Conclusions

OP 350 is a cytotoxic contrast medium, which shows significant genotoxic effects on mouse bone marrow stem cells and there

is a significant reduction in MI and significant increasing in CA and MN caused by OP 350 alone or within X-ray. Both OP 350 and X-ray as a combined treatment demonstrated a very aggressive effects on mouse stem cells, which may belongs to the interact action of both agents.

References

1-Thomson, K.R. and Varma, D. K.(2010). Safe use of radiographic contrast media. Aust. Pres. 33:19-22.

2-Belle, M.B.B., Leffa, D.D., Mazzorana D. and De Andrade V.M.(2013). Evaluation of the mutagenic effect of the iodinated contrast medium Urografina® 292 using the micronucleus test in mouse bone marrow cells. A. of the Brazil. Acad. of Scien.85:737-744.

3-Omnipaque (iohexol) injection products insert. (2010). Princeton, NJ: GE Healthcare.

4-Katayama H., Yamaguchi K., Kozuka T., Takashima T., Seez P. and Matsuura K.(1990). Adverse reactions to ionic and nonionic contrast media: areport from the Japanese Committee on the safety of contrast media. Radiol. 175:621-8.

5-Cochran S.T. and Bomyea K. (2002). Trends in adverse events from iodinated contrast media. AcadRadiol .9:65-8.

6-Ameerunnisa, David, C.M., Savitha G., Ramnarayan B.K. and Sanjay, C.J. (2011). Analysis of cytogenetic effects of radiation in dental personnel exposed to diagnostic X-rays.Int J Hum Genet. 11: 271-276.

7-Borgsdorf, L.R. *et al* (eds) (2005). Drug facts and comparisons 2006, 60th ed. St. Louis, MO: Wolters Kluwer Health, pp2647–2654.

8-Parvez Z, Kormano M, Satokari K, Moncada R and Eklund R.(1987). Induction of mitotic micronuclei by X-ray contrast media in human peripheral lymphocytes. Mut. Res.188: 233-239.

9-Norman A, Cochran J and Sayre W.(2001). Meta-analysis of increases in micronuclei in peripheral blood lymphocytes after angiography or excretory urography. Rad. Res.155: 740–743.

10-Cochran S.T. and Norman A. (1994). Chromosome damage from nonionic contrast media. Invest.Radiol. 29:S206-S207.

11-Namasivayam S, Kalra MK, Torres WE and Small WC. (2006). Adverse reactions to intravenous iodinated contrast media: a primer for radiologists. Emerg.Radiol .12: 210-215.

12-Liu J., Tian J., Liang J., Li X., Yang X., Chen X. *et al*.(2011). Detecting metastasis of gastric carcinoma using high-resolution micro-CT system: *in vivo* small animal Study. Proc. of SPIE. 7965: 79650L-6.

13-Venkataraman A, Alirezaie J, Babyn P and Ahmadian A. (2014). Multi dose computed tomography image fusion based on hybrid sparse methodology. ConfProc IEEE Eng Med Biol Soc. 3901-3904.

14-Allen, J. W.; Schuler, C. F.; Mendes, R. W. and Latt, S. A. (1977). Asimplified technique for in vivo analysis of sister chromatid exchange using 5-bromodeoxyuridine tablets. Cyto. 18:231-237.

15-Gosh, B. B.; Talukder, G. and Shorama, A. (1991). Effect of culture media on spontaneous residence of mitotic index, chromosomal aberration, micronucleus counts, sister chromatid exchange and cell cycle. Kinetics in principle blood lymphocytes of male and female donars. Cyto bios. 67: 71-75.

16-Schmid, W. (1975). The micronucleus test. Mut. Res.31:9-15.

17-Arroyo-Helguera O., Rojas E., Delgado G. and Aceves C.(2008). Signaling pathways involved in the antiproliferative effect of molecular iodine in normal and tumoral breast cells: evidence that 6-iodolactone mediates apoptotic effects. Endo.-Rel. Canc.15:1003–1011.

18-Liu J. , Wang H., Qu A., Li J., Zhao Y. and Wang J.(2013). Combined effects of C225 and 125-iodine seed radiation on colorectal cancer cells. Rad. Onco. 8:1-219.

19-Norman A, Adams FH and Riley RF. (1978). Cytogenetic effects of contrast media and triiodobenzoic acid derivatives in human lymphocytes. Radiol .129: 199-202.

20-Aceves C, Anguiano B and Delgado G.(2005). Is iodine a gatekeeper of the

integrity of the mammary gland?.J. of Mamm. G. Biol. and Neopl. 10: 189–196.

21-Upadhyay G, Singh R, Sharma R, Balapure AK and Godbole MM. (2002). Differential action of iodine on mitochondria from human tumoral-and extra-tumoral tissue in inducing the release of apoptogenic proteins. Mitoch. 2: 199–210.

22-Konig K and Baisch H.(1980). DNA synthesis and cell cycle progression of synchronized L-cells after irradiation in various phases of the cell cycle. Rad. E. Bio. 18: 257.

23-Buckton KE, Evans HJ.(1973). Methods for the analysis of human chromosome aberrations. Geneva: W. H. Organ.p: 21-2.

24-Coggle JE and Noakes GR.(1983). Biological effects of radiation - Radiation and cancer, 2nd ed. London: Taylor & Francis Ltd.. p:111-25, 147-76.

25-Howarth D, Epstein M, Lan L, Tan P and Booker J.(2001). Determination of the optimal minimum radioiodine dose in patients with Graves' disease: a clinical outcome study. Eur J Nucl Med .28: 1489-1495.

26-Sgouros G, Kolbert K and Sheikh A.(2004). Patient-specific dosimetry for 131I thyroid cancer therapy using 124I PET and 3-dimensional-internal dosimetry (3D-ID) software. J Nucl Med. 45: 1366-72.

27-Popova L, Hadjidekova V, Karadjov G, Agova S, Traskov D and Hadjidekov V.(2005). Cytogenetic analysis of peripheral blood lymphocytes after arteriography (exposure to X-rays and contrast medium). RadiolOncol.39: 153-158.

28-Deimling LI, Machado F, Welkerb AG, Peres LM and Mello S.(2008) Micronucleus induction in mouse polychromatic erythrocytes by an X-ray contrast agent containing iodine. Mut. Res.672: 65-68.

29-Adams FH, Norman A, Mello RS and Bass D.(1977). Effect of radiation and contrast media on chromosomes. Preliminary report.Radiol. 124:823-6.