RESEARCH ARTICLE

Evaluation of the Possible Protective Effect of Fisetin against Cyclophosphamide-induced Genotoxicity in Bone Marrow and Spleen Cells of Male Rats

Amani Jabber*, Nada N. Al-Shawi, Ali F. Hasan

Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Received: 10th January, 2023; Revised: 20th March, 2023; Accepted: 28th April, 2023; Available Online: 25th June, 2023

ABSTRACT

Fisetin is a plant flavonoid found in strawberries and other fruits and vegetables such as apples, persimmons, and onions. It has many pharmacological effects like anti-inflammatory, antioxidant, cardioprotective, neuroprotective, and anti-carcinogenicity which are attributed to its ability to reduce oxidative stress which considers the main reason for different disease conditions. Genotoxicity refers to the genetic material destruction within the cell which can be caused by different chemicals as well as radiation. The present study evaluates the effect of orally-administered fisetin daily for seven constitutive days on genotoxicity induced by cyclophosphamide in rats' bone marrow and spleen cells. Results showed that fisetin exhibited a non-significant increase in total chromosomal aberrations, mitotic index, and micronucleus appearance in comparison with the same parameters in control group rats ($p>0.05$); and it produces protection when administered before cyclophosphamide by causing significant decrease ($p<0.05$) in the total chromosomal aberrations, chromatid breaks, ring chromosome, and chromosomal breaks in BM cells and total chromosomal aberrations and chromosomal breaks in spleen cells were shown. In conclusion, fisetin has no genotoxic effect on bone marrow and spleen cells when orally administered alone to rats, and it exhibits some protection against cyclophosphamide-induced genotoxicity.

Keywords: Fisetin, Cyclophosphamide, Genotoxicity, Chromosomal aberrations.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.2.03

How to cite this article: Jabber A, Al-Shawi NN, Hasan AF. Evaluation of the Possible Protective Effect of Fisetin against Cyclophosphamide-induced Genotoxicity in Bone Marrow and Spleen Cells of Male Rats. International Journal of Drug Delivery Technology. 2023;13(2):468-473.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The bone marrow (BM) is a semi-solid tissue found within the spongy (cancellous bone),[1] and it is the primary source for the development of blood and plasma components (hematopoiesis), including myeloid and lymphoid progenitor cells.[2]

Cyclophosphamide (CP) is an alkylating chemotherapeutic drug widely used to treat many cancer types like breast, ovarian, lymphoma, lung carcinomas, and leukemia.[3] Furthermore, it produces toxic effects on many organs, including BM, heart, gonadal and bladder.[4] It has been found to induce any genotoxic effects, including micronuclei, dominant lethal mutation, generation of reactive oxygen species (ROS) that cause DNA damage, sister chromatid exchanges, gene mutations and chromosomal aberrations as mentioned in other research.[5] Moreover, CP is metabolized to the active form 4-hydroxy-cyclophosphamide (4-OHCP),[6,7] which is rapidly decomposed to phosphoramide mustard and acrolein[8] within cells; furthermore, acrolein can enter the cell and interact with the DNA leading to impairment in their synthesis within the cells by generation of ROS and nitric oxide ($NO^{\bullet}$) production that lead to peroxynitrite ($ONOO^{-}$)formation which cause lipids, proteins, and DNA damage inside the cell.[9]

Genotoxicity refers to the genetic materials destruction inside the cell that can lead to mutagenesis or the progress of cancer,[10] and can be caused by different chemical substances as well as radiation.[11] Additionally, *in-vivo* genotoxicity assessment tests are designed to detect compounds that can directly or indirectly lead to genetic damage by utilizing different mechanisms.[12] Chromosomal aberrations are modifications in the genetic materials that can happen through gain, loss or particular segments' rearrangement;[13] and can appear as a consequence of reaction of a genotoxin with the DNA and cause DNA damage (DNA breaks).[14] Furthermore, structural rearrangements in chromosomes may sometimes occur during the normal course of the life cycle but rise due to exposure to mutagenic agents like radiation and stress conditions, including enormously elevated temperature.[15,16]

**Author for Correspondence: dr.amani.j90@gmail.com*

Divers plant extract is reported to have some protection against toxicity induced by chemotherapy.[17] Several clinical trials together with different *in-vitro* and *in-vivo* epidemiological studies illustrated that flavonoids can play a vital role as antioxidant, antimicrobial, anti-allergic, anti-mutagenic and anti-inflammatory agents in treating various diseases.[18]

Fisetin (2-(3,4-dihydroxyphenyl)-3,7-dihydroxychromen-4-one), a flavonoid plant which present in some vegetables and fruits like apples, strawberries, onions, and persimmons;[19] and it is effective as an antioxidant, anti-inflammatory, cardioprotective, neuroprotective and anti-carcinogenic agent which may be related to its role in the reduction of oxidative stress (OS) condition generated in different diseases and modulation of some signaling pathways within the cell.[20]

Objective

The current study is intended to evaluate the fisetin 'possible protection against genotoxicity produced by cyclophosphamide in BM and spleen cells of male albino rats.

MATERIALS AND METHODS

Chemicals and Their Suppliers

Cyclophosphamide (CP) vial 500 mg purchased from Baxter, Germany; fisetin pure powder purchased from Hangzhou Hyper Chemicals Limited, China; colchicine pure powder purchased from Shaanxi Yuantai Biological Technology company, China; dimethyl sulfoxide (DMSO) and diethyl ether purchased from Alpha chemika company, India; fetal bovine serum purchased from Capricorn Scientific, Germany.

Experimental Protocol

Total of 40 healthy male albino rats were taken from the Animal House College of Pharmacy, University of Baghdad, weighing 150 to 220 gm and maintained in the Animal House under a controlled temperature 20 ± 5ºC, and fed commercial pellets and the tap water *ad libitum*. This study was approved by the Ethical Committee of the Department of Pharmacology and Toxicology and the College of Pharmacy, University of Baghdad Scientific Committee. The rats utilized in this work were randomly divided into two parts (20 animals/each) [Part one for assay of chromosomal aberrations and mitotic index (MI), and part two for micronucleus index (MN) assay] and in each experiment, animals were divided to 4 groups (with 5 animals in each) as follows:

Group I: Rats with oral administration of diluted 1% DMSO solution via oral gavage for 7 days; this group represents the negative control.

Group II: Rats orally administered fisetin at a dose (10 mg/kg/day)dissolved in diluted dimethyl sulfoxide 1% (DMSO) for 7 consecutive days. This group represents the positive control group.[21]

Group III: Rats with a single intraperitoneal (IP) injection of cyclophosphamide (CP) (150 mg/kg).[22]

Group IV: Rats orally administered fisetin at a dose (10 mg/kg/day) [dissolved in dimethyl sulfoxide (DMSO)] for 7 consecutive days via gavage tube and a single IP injection of (150 mg/kg) of CP administered on day 7.

On day eight, euthanization of experimental rats was done on day 8 at 9 am by the utilization of diethyl ether; and then to be utilized for the following:

Part One: Evaluation of chromosomal aberrations and the mitotic index (MI) in BM and spleen cells

Total 20 rats were given IP injection of colchicine at a dose of 3.5 mg/kg[23] two hours before scarifying by cervical dislocation, and then aspirated BM cells samples taken from the femur bone were treated using a sterile technique; and spleen samples were also utilized for the evaluation of total chromosomal aberrations, chromosomal structures, and MI.[24]

Part Two: Evaluation of micronucleus (MN) index in BM cells

Total 20 rats were euthanized by the utilization of diethyl ether and the sacrification done by cervical dislocation, femur bone samples were processed and cleaned from other tissue and muscles, and a slide was prepared as mentioned elsewhere,[25] then the slide was checked for the presence of micronucleus formation in at least 1000 bone marrow cells by utilizing oil immersion lens (100x).

Statistical Analysis

The data was analyzed by utilizing a computerized statistical package for the social sciences (SPSS) version 26. Means of groups were compared using the one-way statistical analysis of variance (ANOVA) method followed by Post hoc (Tukey), and the data were stated as mean ±standard deviation (SD) with a significant *p*-value less than 0.05 (<0.05).

RESULTS

In Table 1, rats orally-administered fisetin/positive control (Group II), there were non-significant differences ($p>0.05$) in the total and individual chromosomal aberrations in BM cells compared to the negative control, Group I rats. A single dose of CP injected Intraperitoneally (150 mg/kg) to rats of group III increase the total chromosomal aberrations and ring chromosomes in BM cells significantly ($p<0.05$); but the differences in the individual aberrations in BM cells (chromatid break, chromatid gab, acentric chromosome, dicentric chromosome, chromosomal deletions, chromosomal breaks, and chromosomal gaps) were non-significant ($p>0.05$) as compared with those in the negative control rats/Group I (Table 1).

Furthermore, Table 1 showed that there is a significant decrease ($p<0.05$) in total chromosomal aberrations, chromatid breaks, ring chromosome, and chromosomal breaks in BM cells of group IV rats (fisetin and CP); but the differences were non-significant ($p>0.05$) in the chromatid gab, acentric chromosome, dicentric chromosome, chromosomal deletions, and chromosomal gaps compared with aforementioned aberrations in group III rats.

Also, Table 1 showed that an orally-administered dose of fisetin (10 mg/kg/day) for 7 days (positive control-Group II) caused the MI in BM cells to non-different significantly ($p>0.05$) in comparison with such index in BM cells of negative control (Group I) rats.

While a single intraperitoneal dose of CP in rats of (Group III) decreased the MI in BM cells significantly ($p<0.05$) as compared to such index in negative control-group I rats (Table 1).

Moreover, Table 1 also showed that in rats of group IV (fisetin and CP), the increase was a non-significantly ($p>0.05$) in the MI in BM cells compared with the corresponding index in rats of group III.

In Table 2, in group II-positive control rats, the differences were non-significant ($p>0.05$) in the total and individual chromosomal aberrations in spleen cells compared to those in negative control group I rats.

Meanwhile, single dose of CP injected IP (150 mg/kg) (Group III), exhibited an increase in total chromosomal aberrations and individual chromosomal aberrations in spleen cells but not significant ($p>0.05$) in comparison with that of group I. Furthermore, as shown in Table 2, the decrease in the total chromosomal aberrations and chromosomal breaks in spleen cells of group IV rats(fisetin before CP) was significant ($p<0.05$) as compared to rats of group III; while the differences were non-significant ($p>0.05$) in the individual aberrations (chromatid gab, chromatid break, acentric chromosome, dicentric chromosome, chromosomal deletions, ring chromosome, and chromosomal gaps) as compared with such aberrations in CP injected rats ($p>0.05$).

Additionally, Table 2 showed that orally-administered fisetin at a dose (10 mg/kg/day) (Group II-positive control) exhibited a non-significant change ($p>0.05$) in the MI in spleen cells in comparison with such index in spleen cells of negative control-Group I; while a single dose of CP injected IP in rats of group III decrease the MI in spleen cells more significantly ($p<0.05$) in comparison with that index of negative control-Group I rats.

Furthermore, in rats of group IV, the MI in spleen cells was non-significantly different ($p>0.05$) as compared to that index in rats of Group III (Table 2).

Table 3 showed that in group II rats (orally-administered fisetin at a dose of 10 mg/kg/day, positive control), there were non-significant differences ($p>0.05$) in the MN index in BM cells compared to the corresponding index in BM cells of negative control (Group I) rats. Furthermore, Table 3 revealed a statistically significant increase ($p<0.05$) in the micronucleus index in BM cells in group III rats compared to such index in negative control (Group I) rats.

Also, Table 3 showed that the MN index in BM cells of group IV (fisetin and CP) was non-significantly different ($p>0.05$) in rats compared to that index in BM cells of group III rats.

Data are expressed as mean ± S.D; n=7 animals in each group

- Values with different small letters (a, and b) are significantly different ($p<0.05$).
- Values with the same small letter (a) in each of the bone marrow (BM) and spleen cell columns are different non-significantly ($p>0.05$).
- Values with the (bc) letters represented as b= different non-significantly from group III ($p>0.05$) and c= different significantly from groups I and II ($p<0.05$).
- Values with both symbol (#) and the letter (b) [#b] [#= considered as different non-significantly from groups I and II ($p>0.05$) and b= different significantly from groups III ($p<0.05$).

Data are expressed as mean ± S.D; n=7 animals in each group

- Values with different small letters (a, and b) are significantly different ($p<0.05$).
- Values with the same small letter (a) in each of the bone marrow (BM) and spleen cell columns are different non-significantly ($p>0.05$).
- Values with the (bc) letters represented as b= different non-significantly from group III ($p>0.05$) and c= different significantly from groups I and II ($p<0.05$).
- Values with both symbol (#) and the letter (b) [#= consider as different non-significantly from groups I and II ($p>0.05$) and b= different significantly from groups III ($p<0.05$).
- Data are expressed as mean ± SD by one-way ANOVA test.
- Values with different small letters superscripts (a, and b) in BM cells are different significantly ($p<0.05$).
- Values with the same small letters superscripts (a and b) in BM cells are different non-significantly ($p>0.05$).

DISCUSSION

There were no previous studies have been made to detect the genotoxicity of fisetin in rats. For this reason, this study was designed to evaluate the protective effect of fisetin against CP-induced chromosomal aberrations through the assessment of MI and MN appearance in male albino rats. Results of this study showed that administration of fisetin orally to rats exhibited non-significant change ($p>0.05$) in total chromosomal aberrations, individual chromosomal aberrations, MI, and MN appearance compared to control-Group I.

Furthermore, the present study (Tables 1 and 2) showed a significant decrease ($p<0.05$) in the MI in BM and spleen cells of rats injected with CP (Group III) compared with such index in both organs in the control group. The aforementioned index gives an indication of cell division, where the decrease in the MI (in this study) and consistent with those of others; this, may be referred to a delay in the kinetics of cell proliferation and supposed to be a result of drug cytotoxicity or genotoxicity on mitosis at different dose.[26]

Furthermore, total chromosomal aberrations in BM and spleen cells were significantly-increased ($p<0.05$) after CP injection as compared with the negative control group, and this result agrees with what was mentioned in the study of Kour J (2017).[27] Besides, the MN assay is recognized as one of the most successful and reliable assays for genotoxic carcinogens.[28] This study also showed that IP injection of CP caused a statistically significant increase in MN appearance compared with such appearance in the negative control group; where such appearance is an important parameter that can be utilized to detect the destruction of chromosomes and this confirms the damage to the genetic material by CP in somatic cells by induction of chromosomal aberrations, micronuclei, chromatid exchanges, and gene mutations.[27,29]

Table 1: Individual and total chromosomal aberrations in bone marrow cells of rats treated with fisetin alone and pretreatment with cyclophosphamide

Bone marrow cells	*Chromatid break*	*Chromatid gab*	*Acentric chromosome*	*Dicentric chromosome*	*Ring chromosome*	*Deletions*	*Chromosome break*	*Chromosome gab*	*Total chromosomal aberrations*	*Mitotic index*
Control group I	0.066 ± 0.0089 [a]	0.07 ± 0.01 [a]	0.254 ± 0.035 [a]	0.21 ± 0.046 [a]	0.214 ± 0.011 [a]	0.04± 0.0122 [a]	0.074 ± 0.0151 [a]	0.066 ± 0.0151 [a]	0.12425±0.0085 [a]	8.16 ± 0.8117 [a]
Fisetin 10 mg/kg group II	0.058 ± 0.0083 [a]	0.06 ± 0.015 [a]	0.244 ± 0.025 [a]	0.2 ± 0.025 [a]	0.208 ± 0.008 [a]	0.034 ± 0.0134 [a]	0.066 ± 0.0151 [a]	0.06 ± 0.01 [a]	0.11625±0.0066 [a]	7.92 ± 0.7496 [a]
Cyclophos-phamide 150 mg/kg group III	0.078 ± 0.0130 [a]	0.086 ± 0.024 [a]	0.284 ± 0.0687 [a]	0.22 ± 0.043 [a]	0.252 ± 0.0449 [b]	0.05 ± 0.0254 [a]	0.088 ± 0.0130 [a]	0.084 ± 0.028 [a]	0.14275±0.0166 [b]	6.76 ± 0.7861 [b]
Fisetin and cyclophos-phamide group IV	0.062 ± 0.0130[#b]	0.062 ± 0.031 [a]	0.254 ± 0.0391 [a]	0.194 ± 0.011 [a]	0.214 ± 0.011 [a]	0.036 ± 0.0151 [a]	0.056 ± 0.0391 [#b]	0.062 ± 0.0356 [a]	0.1175± 0.0029 [a]	7.16 ± 0.6767 [bc]

Table 2: Individual, total chromosomal aberrations and mitotic index in spleen cells of rats treated with fisetin alone and pretreatment with cyclophosphamide

Spleen cells	*Chromatid break*	*Chromatid gab*	*Acentric chromosome*	*Dicentric chromosome*	*Ring chromosome*	*Deletions*	*Chromosome break*	*Chromosome gab*	*Total chromosomal aberrations*	*Mitotic index*
Control group I	0.054 ± 0.016 [a]	0.066 ± 0.011 [a]	0.206 ± 0.018 [a]	0.186 ± 0.015 [a]	0.208 ± 0.019 [a]	0.034 ± 0.011 [a]	0.066 ± 0.0134 [a]	0.052 ± 0.0192 [a]	0.109 ± 0.0082 [a]	6.96 ± 0.2073 [a]
Fisetin 10mg/kg group II	0.048 ± 0.008 [a]	0.058 ± 0.013 [a]	0.2 ± 0.012 [a]	0.182 ± 0.008 [a]	0.198 ± 0.010 [a]	0.03 ± 0.012 [a]	0.058 ± 0.0083 [a]	0.05 ± 0.0158 [a]	0.103 ± 0.0039 [a]	7.24 ± 0.4505 [a]
Cyclophosphamide (CP) 150mg/kg group III	0.06 ± 0.029 [a]	0.072 ± 0.014 [a]	0.21 ± 0.015 [a]	0.2 ± 0.045 [a]	0.226 ± 0.040 [a]	0.04 ± 0.01 [a]	0.072 ± 0.0164 [a]	0.072 ± 0.0164 [a]	0.119 ± 0.0124 [a]	6.24 ± 0.2881 [b]
Fisetin prior to CP group IV	0.048 ± 0.008 [a]	0.064 ± 0.013 [a]	0.174 ± 0.087 [a]	0.172 ± 0.013 [a]	0.202 ± 0.014 [a]	0.026 ± 0.008 [a]	0.048 ± 0.0178 [#b]	0.052 ± 0.0148 [a]	0.09825± 0.0077 [#b]	6.74 ± 0.5319 [bc]

Table 3: Incidence of micronucleus (MN) index in bone marrow cells

Animal groups	*Micronucleus index in BM cells*
Control (Group I)	6.9166 ± 0.6431 [a]
Fisetin 10 mg (Group II)	6.6666 ± 0.3559 [a]
Cyclophosphamide (CP) (Group III)	9.0833 ± 0.9453 [b]
Fisetin prior to CP(Group IV)	8.3666 ± 0.6186 [b]

and in fact, acrolein (one of the metabolites of CP) within the cell can rapidly activate the intracellular ROSs and nitric oxide production which leads to the formation of the peroxynitrite causing lipids, proteins, and DNA damage inside the cell.[30]

Moreover, several studies have been found that ROSs production had a destructive effect on the integrity of chromosome structure and the use of an antioxidant substance may reduce it.[31] The current study showed the usefulness of the administration of fisetin before CP; since the decrease in the total chromosomal aberration in BM and spleen cells (Tables 1 and 2) were significant ($p<0.05$) compared to corresponding aberrations in group III-CP, and this may be related to the antioxidant activity of fisetin by scavenging the ROSs that were generated by CP leading to lipid peroxidation and chelating iron.[32] Additionally, from the chemical structure of fisetin, Firuzi O and his colleagues (2005) mentioned that its antioxidant activity is mainly related to the 3-OH group together with the two hydroxyl group at position 3'and 4' and the double bond between the 2- and 3 carbon which are able to enhance the antioxidant activity.[33]

CONCLUSION

According to the results of the present study, it can be concluded that fisetin flavonoid is non- genotoxic in BM and spleen cells of male albino rats when orally administered alone; with some protection against CP-induced genotoxicity.

REFERENCES

1. Farhi DC. Pathology of bone marrow and blood cells. Berghahn Books; 2009.
2. Brown D, Gatter K, Natkunam Y, Warnke R. Bone Marrow Diagnosis: An Illustrated Guide: Second Edition. Bone Marrow Diagnosis: An Illustrated Guide: Second Edition. John Wiley & Sons; 2007. 1–216 p.
3. Khodeer DM, Mehanna ET, Abushouk AI, Abdel-Daim MM. Protective effects of evening primrose oil against cyclophosphamide-induced biochemical, histopathological, and genotoxic alterations in mice. Pathogens. 2020;9(2):98.
4. Emadi A, Jones RJ, Brodsky RA. Cyclophosphamide and cancer: golden anniversary. Nat Rev Clin Oncol. 2009;6(11):638–47.
5. Sekeroğlu V, Aydin B, Sekeroğlu ZA. Viscum album L. extract and quercetin reduce cyclophosphamide-induced cardiotoxicity, urotoxicity and genotoxicity in mice. Asian Pac J Cancer Prev. 2011;12(11):2925–31.
6. KA Mills, R Chess-Williams CM. Novel insights into the mechanism of cyclophosphamide-induced bladder toxicity: chloroacetaldehyde's contribution to urothelial dysfunction in vitro. Arch Toxicol. 2019;93(11):3291–303.
7. Korkmaz A, Topal T, Oter S. Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species as well as PARP activation. Cell Biol Toxicol. 2007;23(5):303–12.
8. Takada K, Arefayene M, Desta Z, Yarboro CH, Boumpas DT, Balow JE, et al. Cytochrome P450 pharmacogenetics as a predictor of toxicity and clinical response to pulse cyclophosphamide in lupus nephritis. Arthritis Rheum Off J Am Coll Rheumatol. 2004;50(7):2202–10.
9. Tripathi DN, Jena GB. Intervention of astaxanthin against cyclophosphamide-induced oxidative stress and DNA damage: a study in mice. Chem Biol Interact. 2009;180(3):398–406.
10. Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, et al. Senescence in premalignant tumours. Nature. 2005;436(7051):642.
11. De Flora S IA. Mutagenesis and cardiovascular diseases: molecular mechanisms, risk factors, and protective factors. Mutat Res Mol Mech Mutagen. 2007;621(1–2):5–17.
12. Smoke T, Smoking I. IARC monographs on the evaluation of carcinogenic risks to humans. IARC, Lyon. 2004;1:1–1452.
13. Theisen A, Shaffer LG. Disorders caused by chromosome abnormalities. Appl Clin Genet. 2010;3:159.
14. Zhao C, Kumar R, Zahlsen K, Bager Sundmark H, Hemminki K, Eide I. Persistence of 7-(2-hydroxyethyl) guanine-DNA adducts in rats exposed to ethene by inhalation. Biomarkers. 1997;2(6):355–60.
15. Kumar G, Tripathi R. Influence of heat stress on genome of grass pea (Lathyrus sativus L.). J Environ Biol. 2009;30(3):405–8.
16. Mrózek K, Bloomfield CD. Chromosome aberrations, gene mutations and expression changes, and prognosis in adult acute myeloid leukemia. ASH Educ Progr B. 2006;2006(1):169–77.
17. Jabbar AASA, Kathem SH. The Protective Effect of Mentha spicata Ethanolic Extract on Irinotecan-Induced Mucositis in Mice. Iraqi J Pharm Sci. 2019;28(1).
18. Abubakar MB, Abdullah WZ, Sulaiman SA, Suen AB. A review of molecular mechanisms of the anti-leukemic effects of phenolic compounds in honey. Int J Mol Sci. 2012;13(11):15054–73.
19. Sahu BD, Kalvala AK, Koneru M, Mahesh Kumar J, Kuncha M, Rachamalla SS, et al. Ameliorative effect of fisetin on cisplatin-induced nephrotoxicity in rats via modulation of NF-κB activation and antioxidant defence. PLoS One. 2014;9(9):e105070.
20. Khan N, Syed DN, Ahmad N, Mukhtar H. Fisetin: a dietary antioxidant for health promotion. Antioxid Redox Signal. 2013;19(2):151–62.
21. Sari EN, Soysal Y. Molecular and Therapeutic Effects of Fisetin Flavonoid in Diseases. J Basic Clin Heal Sci. 2020;4(3):190–6.
22. Ghanim WK. Evaluating the Effects of Different Doses of Vitamin B2 and Single Dose of Vitamin B12 Against Myelosuppression Induced by Cyclophosphamide in Experimental Rats. Iraqi J Pharm Sci (P-ISSN 1683-3597, E-ISSN 2521-3512). 2020;29(1):134–42.
23. Wongpa S, Himakoun L, Soontornchai S, Temcharoen P. Anti-mutagenic effects of piperine on cyclophosphamide-induced chromosome aberrations in rat bone marrow cells. Asian Pacific J Cancer Prev. 2007;8(4):623–7.
24. JW Allen, CF Shuler, RW Mendes SL. A simplified technique for in vivo analysis of sister-chromatid exchanges using 5-bromodeoxyuridine tablets. Cytogenet Genome Res. 1977;18(4):231–7.
25. Fenech M. The in vitro micronucleus technique. Mutat Res Mol Mech Mutagen. 2000;455(1–2):81–95.
26. Amirthalingam T, Velusamy G, Pandian R. Cadmium-induced

changes in mitotic index and genotoxicity on Vigna unguiculata (Linn.) Walp. J Environ Chem Ecotoxicol. 2013;5(3):57–62.
27. Kour J, Ali MN, Ganaie HA, Tabassum N. Amelioration of the cyclophosphamide induced genotoxic damage in mice by the ethanolic extract of Equisetum arvense. Toxicol reports. 2017;4:226–33.
28. Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutat Res Toxicol Environ Mutagen. 2003;534(1–2):65–75.
29. Krishna G, Petrere J, Anderson J, Theiss J. Use of cyclophosphamide as a positive control in dominant lethal and micronucleus assays. Mutat Res Mutagen Relat Subj. 1995;335(3):331–7.
30. Jeelani R, Khan SN, Shaeib F, Kohan-Ghadr H-R, Aldhaheri SR, Najafi T, et al. Cyclophosphamide and acrolein induced oxidative stress leading to deterioration of metaphase II mouse oocyte quality. Free Radic Biol Med. 2017;110:11–8.
31. Nardi NB, Meirelles L. Mesenchymal stem cells: isolation, in vitro expansion and characterization. Stem Cells. 2008;249–82.
32. Vorhees C V, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006;1(2):848–58.
33. Firuzi O, Lacanna A, Petrucci R, Marrosu G, Saso L. Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. Biochim Biophys Acta (BBA)-General Subj. 2005;1721(1–3):174–84.