Assessment the Protective Role of Vitamin C on the Genotoxicity of 5-Fluorouracil in Male Albino Mice

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Abstract: The present study was carried out to evaluate the possible prophylactic role of vitamin C against the genopathogenicity of 5-fluorouracil (5-FU) on bone marrow chromosomes of adult male albino mice. Sixty adult mice were used in the present study. They were allocated into six equal groups. The first group served as the control group. Four groups received the suggested dose of 5-FU (80mg/kg b.wt.) and was given (i.p.) for two and four weeks, every other day, alone and concurrently daily injected (i.p.) with vitamin C (12mg/kg b.wt). Both doses were calculated according to the equivalent therapeutic dosages of human-mouse conversion factor. The sixth group injected with 12mg/kg b.wt. vitamin C (i.p.) daily for four weeks. Four varieties of structural chromosomal aberrations were detected in this study, exhibiting statistical highly significant increase ($P<0.001$) in comparable with the control group. The results of total chromosome abnormalities were significantly time-dependent manner *in vivo*. Such aberrations were markedly inclined after vitamin C, separately or in 5-FU associated mode, recording significant decrease ($P<0.05$). The study suggested that vitamin C has a relatively protective role on 5-FU induced chromosomal aberrations. In conclusion, vitamin C may be considered as a potential protective agent against genotoxicity of 5-FU on bone marrow cells of albino mice (*Mus musculus*).
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Key words: 5-FU, Vitamin C, Structural chromosomal aberrations, Bone marrow, Albino mice.

1. Introduction

Most plants and animals synthesize ascorbic acid from D-glucose or D-galactose in liver for their own requirement **(Haworth** and **Hirst, 1993**). However, apes and humans can't synthesize ascorbic acid due to lack of an enzyme gulonolactone oxidase (**Naidu, 2003**). Hence, ascorbic acid has to be supplemented mainly through fruits, vegetables and tablets.

Vitamin C (ascorbic acid, ascorbate) is a major water soluble antioxidant with a variety of biological functions. It plays roles in controlling the oxidative stress **(Panayiotidis** and **Collins, 1997)**, collagen and carnitine synthesis and may be important in maintaining proper immune cell function **(Goldschmidt, 1991** and **Penn *et al*., 1991)**. In rodents, vitamin C protects normal tissues from the oxidative damage associated with chemotherapy and radiation **(Fujita *et al*., 1982** and **Okunieff** and **Suit, 1987)**. These and other observations have created interest in using vitamin C as an adjuvant treatment for cancer **(Henson *et al*., 1991)**. So this vitamin can protect DNA against damages induced by various chemicals **(Duthie *et al.,* 1996** and **Blasiak and Kowalik, 1999)**.

Vitamin C accumulates in solid tumours at concentrations higher than those in surrounding normal tissue **(Langemann *et al*., 1989** and **Agus *et al*., 1999)**. This has raised concerns that vitamin C may provide tumours with antioxidant protection from traditional therapeutic modalities **(Raloff, 2000)**. However, vitamin C may be useful as an anti-cancer agent if cytotoxic ascorbate concentrations can be achieved in tumours **(Riordan *et al*., 1995)**.

5-fluorouracil (5-FU) is an antimetabolite chemotherapy drug and clinically commonly used for the treatment of solid tumours of the breast, ovaries, head, neck, gastrointestinal and colorectal tumors **(Heidelberger, 1974; Kovach** and **Beart, 1990; Chu *et al*., 2003; Alvarez-Cabellos *et al*., 2007; Sablin *et al*., 2009** and **Clark *et al*., 2012)**.

The chemotherapeutic drug of 5-FU is a pyrimidine analog, has a stable fluorine atom in place of a hydrogen atom at position 5 of the uracil ring **(Heidelberger, 1974; Wohlhueter *et al.*, 1980** and **Clark *et al*., 2012)**. This antimetabolite drug (5-FU) mimics uracil and is incorporated into RNA and DNA, where it inhibits the thymidylate synthetase enzyme necessary for DNA synthesis **(Heidelberger *et al*., 1957** and **Chu *et al*., 2003)**.

5-FU *per se* is devoid of antineoplastic activity **(Pinedo** and **Peters, 1988** and **Clark *et al*., 2012)**. It enters the cell through a carrier-mediated transport system and is converted to the corresponding deoxynucleotide 5-flurodeoxyuridine monophosphate (5-FdUMP), which competes with deoxyuridine monophosphate for thymidylate synthase. 5-FdUMP acts as a pseudosubstrate and is trapped with the enzyme and its coenzyme N^5, N^{10} -methylene tetrahydrofolic acid (leucovorin), in a ternary

complex that cannot proceed to release products **(Pinedo** and **Peters, 1988** and **Clark *et al*., 2012)**.

So, in order to express a cytotoxic effect of 5-FU, it has to be converted to one of its active metabolites, 5-fluorouridine 5'-triphosphate (FuTP), 5-fluoro-2'-deoxyuridine-5'monophosphate (FduMP), or 5-fluorodeoxyuridine-5'triphosphate (FduTP) **(Pinedo** and **Peters, 1988)**. In turn, the cytotoxic action of 5-FU in most systems was attributed primarily to its anabolism to 5-fluoro-2'-deoxyuridine monophosphate (FduMP), a potent inhibitor of thymidylate synthase **(Schwartz *et al.*, 1995** and **Evrard *et al.*, 1999)**, which is a pivotal enzyme in pyrimidine biosynthesis **(Hartmann** and **Heidelberger, 1961** and **Yeh *et al.*, 1998)**.

In this concern, the liver and various other tissues DPD enzyme catabolizes 5-FU into 5,6-dihydro-5-fluorouracil **(Diasio** and **Harris, 1989** and **Clark *et al*., 2012)** and finally leading to the formation of α-fluoro-β-ureidopropionic acid and α-fluoro-β-alanine (FBAL) **(Diasio** and **Harris, 1989)**. Such suggestion was supported by observation of most patients tolerate 5-FU reasonably well, a number of cancer patients with DPD deficiency were shown at increased risk for severe toxicities, including diarrhea, mucositis, and neurotoxicity, as well as death, after administration of standard doses of 5-FU **(Saif *et al.*, 2009)**.

On the other hand, **Farczádi *et al.* (2003)** found that the given short-term cytostatic therapy of 5-FU 600 mg/m^2 for 2 days in twenty patients with rectal adenocarcinoma was detected a significant increase of the apoptotic index and a non-significant decrease of the mitotic index.

Bach *et al.* (2006) recorded that the 5-FU produced a prompt decrease in the mitotic index at 6 h with complete cessation of almost all measured activity up to 72 h at which point recovery occurred in the murine gut.

Gu *et al.* (2012) used 5-FU as a cytotoxic drug to chemoresistance of human hepatocellular carcinoma, causing lowering of mitotic index in human cell line with an increased proportion in S and G2/M phases with a concomitant decrease in G0/G1 phase.

Furthermore, in **(2013), Kuznietsova *et al.*** recorded that the number of tumour node and tumour total area under the influence of 5-FU were decreased by 40-54%.

2. Materials and Methods

Experimental Animals:

The present study was carried out on the male Swiss albino mice of CD-1 *(Mus musculus)* with an average age of 12 weeks and body weight of ~ 25 g. The animals were obtained from Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt.

The animals were housed in cages and fed *ad libitum* with a standard diet and provided with free access to water, being kept under suitable laboratory conditions during the whole period of experimentation and the healthy animals were used.

The Applied Drugs

The drugs used in the present work are 5-fluorouracil (5-FU) and vitamin C.

Vitamin C (ascorbic acid) is available in the form of ampoules. The drug is supplied in packages enclosing three ampoules, each containing 1000 mg/ 5 ml of vitamin C.

5-FU is available in the form of ampoules. The drug is supplied in packages enclosing five ampoules, each containing 250 mg/ 5 ml of 5-fluorouracil; having the chemical formula ($C4H3FN_2O_2$**).**

Both doses of vitamin C (12mg/kg b.wt.) and 5-FU (80mg/kg b.wt.) were diluted in normal saline solution (0.9% NaCl). Experimented mice were injected intraperitoneally.

The dose of 5-fluorouracil (80mg/kg b.wt.) and the dose of vitamin C (12mg/kg b.wt.) were calculated according to the equivalent therapeutic dosages of human-mouse conversion factor by **Paget** and **Barnes (1964)**, and were given for the desired periods (two and four weeks).

Design of Experimental Animals

Sixty adult mice were allocated into six equal groups of 10 mice each. The first group served as the control group (C group) and the rest four groups divided into F2 and F4 groups; which were 5-FU treated groups, whilst the other two groups FV2 and FV4 groups were act as protective groups. Beside, the last group (V group) was vitamin C-treated group. So, these six groups were designed as the following manner:

Group "1": Each animal was injected intraperitoneally (i.p.) with saline solution (0.9 % NaCl), the solvent of the drug was given in every other day for four weeks **(C group)**.

Group "2": Each animal was injected (i.p.) with 5-FU (80 mg/kg b. wt.) in every other day for two weeks **(F2 group)**.

Group "3": Each animal was injected (i.p.) with 5-FU (80 mg/kg b. wt.) in every other day for four weeks **(F4 group)**.

Group "4": Each animal treated with 80 mg/kg b. wt. of desired drug (5-FU). Each was injected (i.p.) with 5-FU in every other day for two weeks and concurrently daily injected (i.p.) with vitamin C (12mg/kg b. wt.) for two weeks **(FV2 group)**.

Group "5": Each animal treated with 80 mg/kg b. wt. of desired drug (5-FU). Each was injected (i.p.) with 5-FU in every other day for four

weeks and concurrently daily injected (i.p.) with vitamin C (12mg/kg b. wt.) for four weeks **(FV4 group)**.

Group "6": Ten mice were considered as a test for adverse consequences induced in subject to vitamin C alone. Each mouse was injected (i.p.) with daily dose of vitamin C (12 mg/kg b. wt.) for four weeks **(V group)**.

At the end of the experiment, the mice were sacrificed by severing their neck blood vessels, immediately dissected and their bone marrow and processed for microscopic preparations.

Mitotic index was calculated by counting the divided cells among at least 1000 metaphase spreads/animals group (n=5) and expressed in percentage.

Bone Marrow Chromosomal aberration assay

The technique as given by **Lee** and **Elder (1980)** and modified **Baker *et al.* (1982)** was employed in the present study. Observation was made using bright field and photographs were taken with a 100 X oil objective lens. 500 well spread metaphases from each group of animals were examined.

Statistical analysis of the data of chromosomal aberrations was carried out by *t*-test, SPSS statistics 17.0.

3. Results

In short, these different cases are listed as follows:

A) Mice Treated with (80 mg/kg body weight) of 5-fluorouracil in Every other Day (Figs. 2-14):

1- Animals Examined after Two Weeks of Treatment (F2 group) (Figures. 2-8):

Four varieties of chromosomal aberrations were designated in this group, recording the total mean of (79.8), thus exhibiting a statistically highly significant increase ($P<0.001$) relative to the control group, which recorded the total mean of (2.2). These aberrations were constituted of deletion, centromeric attenuation, fragments (centric and acentric) and ring chromosome recording the means of (35.4, 26, 13.6 and 4.8, respectively). Ring chromosome is regarded to represent a statistical highly significant increase ($P<0.01$), but the other aberrations were regarded to represent a statistically highly significant increase ($P<0.001$). Thus, the highest frequency of the chromosomal aberrations was represented by deletions (35.4) at this group.

2- Animals Examined after Four Weeks of Treatment (F4 group) (Figs. 9-14):

Four varieties of chromosomal aberrations were also detected in this group but with a different total mean (80) showing a statistical highly significant increase ($P<0.001$) in comparison with the total mean of control (2.2). These aberrations were also constituted of deletion, centromeric attenuation, fragments (centric and acentric) and ring chromosome recording the means of (2.2, 43.2, 32.6 and 2, respectively). Deletion and ring chromosome aberration represent a statistically significant increase ($P<0.05$), but centromeric attenuation and fragments (centric and acentric) being regarded to represent a statistically highly significant increase ($P<0.001$). Thus, the highest frequency of the chromosomal aberrations was represented (43.2) by centromeric attenuation.

B) Mice Treated with (80 mg/kg body weight) of 5-fluorouracil in Every other Day and Simultaneously Daily with Vitamin C (Figures. 15-18):

1- Animals Examined after Two Weeks of Treatment (FV2 group) (Figs. 15 and 16):

In this case only three varieties of chromosomal aberrations were designated in this group, recording decrease in the total mean of (27.2), thus exhibiting a statistically highly significant increase ($P<0.001$) relative to the control group, which recorded the total mean of (2.2). These aberrations were constituted of deletion, centromeric attenuation and fragments (centric and acentric) recording the means of (9.6, 6.4 and 11.2, respectively). Ring chromosomes aberration is represented a statistical significant decrease ($P<0.05$). The centromeric attenuation is recorded a statistical highly significant increase ($P<0.01$), beside deletions and fragments (centric and acentric) are regarded to represent a statistical highly significant increase, but having ($P<0.001$). Thus, the highest frequency of the chromosomal aberrations was represented (11.2) by fragments (centric and acentric).

The three varieties of chromosomal aberrations that were detected in this group with a total mean (27.2) making a statistical highly significant decrease ($P<0.001$) in comparison with the total mean of F2-treated group (79.8) as illustrated in **table (1) figure (22)**.

2- Animals Examined after Four Weeks of Treatment (FV4 group) (Figs. 17, 18):

Three varieties of chromosomal aberrations were also detected in this group but with a different total mean (42) marking a statistically highly significant increase ($P<0.001$) in comparison with the total mean of control (2.2). These aberrations were also constituted of deletion, centromeric attenuation and fragments (centric and acentric) recording the means of (2, 14.4 and 25.6, respectively). Ring chromosome aberration is represented a statistically significant decrease ($P<0.05$), deletion is recorded a statistically highly significant increase ($P<0.01$), but centromeric attenuation and fragments (centric and

acentric) chromosomes are regarded to represent a statistically highly significant increase (P<0.001). Thus, the highest frequency of the chromosomal aberrations was represented by (25.6) fragments (centric and acentric).

The three varieties of chromosomal aberrations that were detected in this group with a total mean (42) marking a statistical highly significant decrease (P <0.001) in comparison with the total mean of F4-treated group (80) as shown in **table (1) figure (22)**.

C) Mice Treated Daily with Vitamin C (12 mg/kg b.wt.) for Four Weeks (Figs. 19 & 20):

Four varieties of chromosomal aberrations were designated in this group, recording the total mean of (3.2), thus exhibiting a statistically insignificant increase (P <0.05) relative to the control group, which recorded the total mean of (2.2). These aberrations were constituted of deletion, centromeric attenuation, fragments (centric and acentric) chromosomes and ring chromosome recording the means of (1.4, 0.4, 0.6 and 0.8, respectively).

The mitotic indices of the entire collected groups in the present study, as shown in **table (2)** and **figure (23)** ranged from a little different average of percentages (38% & 35.7%) between (C & V groups, respectively) to highly elevated average of percentage (20.5% & 38%) between the two groups (F2 & C, respectively). Such results reflected the expected inhibition role of cytostatic agent "5-FU" on mitotic rate of bone marrow and the regulatory role of vitamin C on division stages

Figure (1)

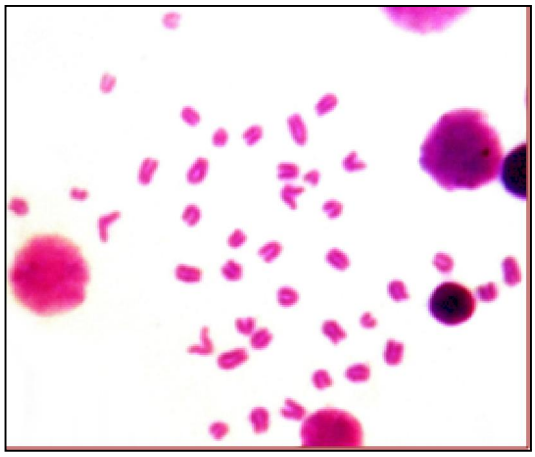


Figure (2)

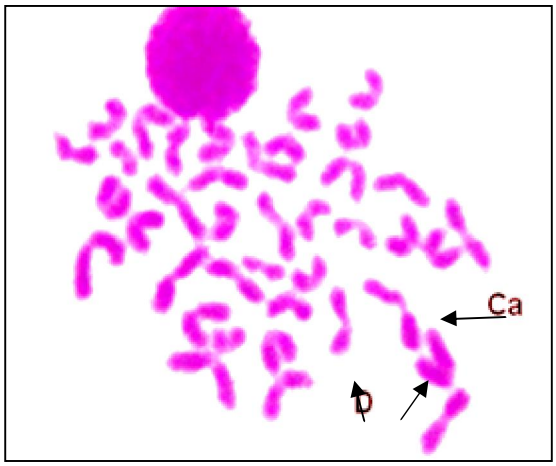
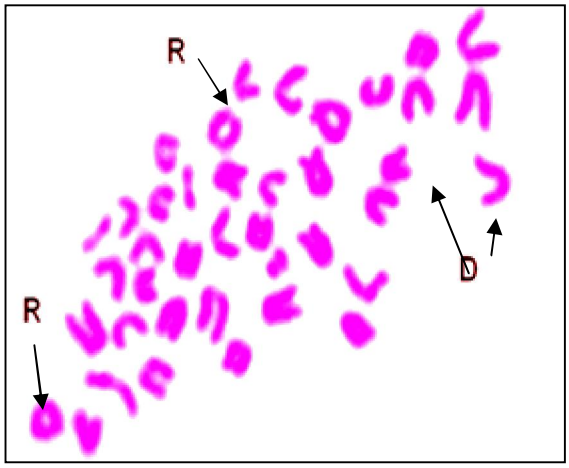


Figure (3)



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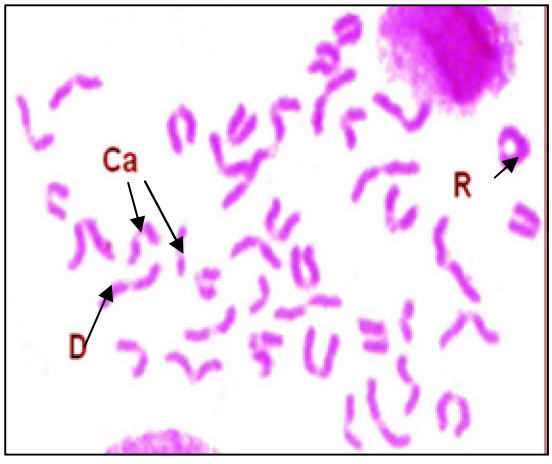


Figure (1): Photomicrograph of metaphase telocentric chromosomes (2n=40) obtained from bone marrow of control male albino mouse, *Mus musculus* (C-group). (X 1000)

Figure (2): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F2-group) exhibiting centromeric attenuation (Ca) and deletions (D). (X 1000)

Figure (3): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F2-group) reflecting ring chromosome (R) and deletions (D). (X 1000)

Figure (4): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F2-group) detecting centromeric attenuation (Ca), deletion (D) and ring chromosome (R). (X 1000)

Fig (5)

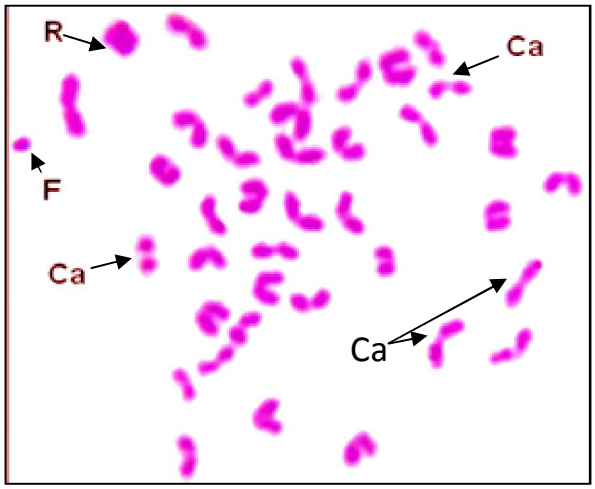


Fig (6)

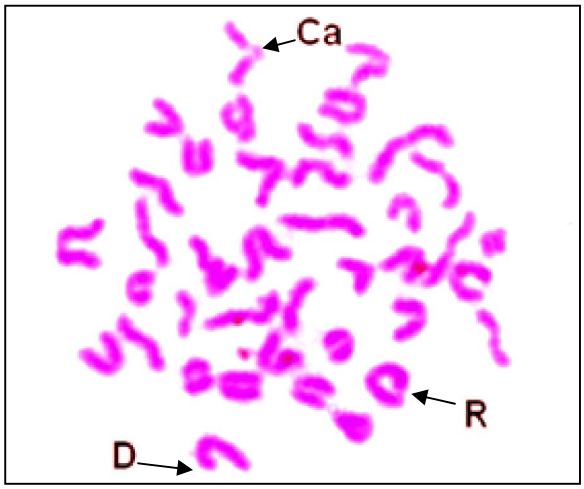
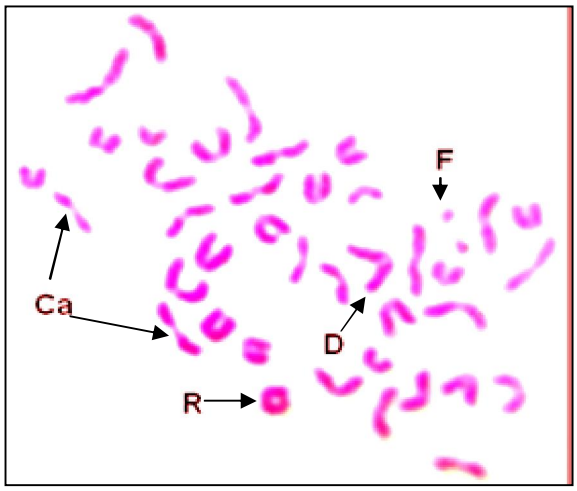


Fig (7)



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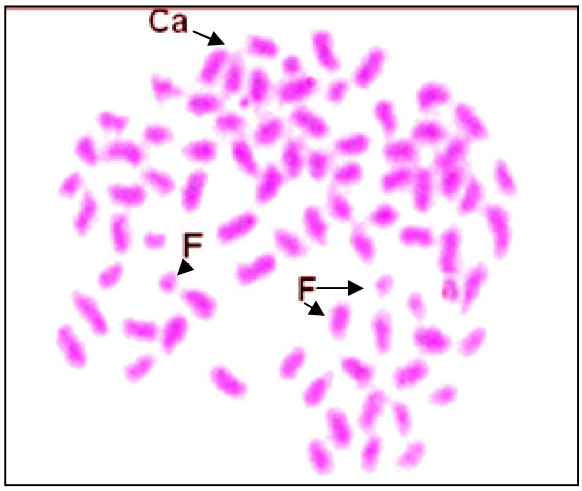


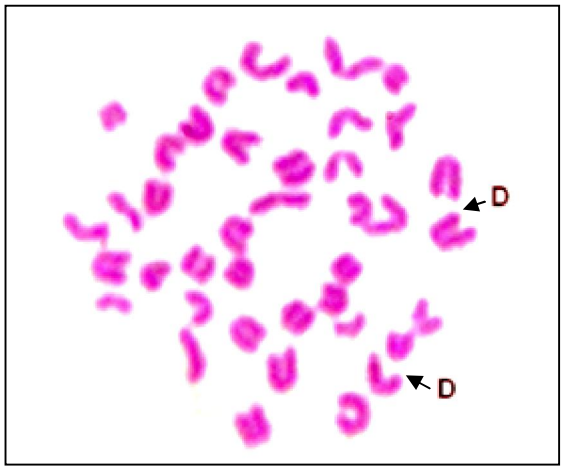
Figure (5): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F2-group) showing ring chromosome (R), centromeric attenuation (Ca) and fragment (F). (X 1000)

Figure (6): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F2-group) detecting centromeric attenuation (Ca), deletion (D) and ring chromosome (R). (X 1000)

Figure (7): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F2-group) reflecting ring chromosome (R), deletion (D), centromeric attenuation (Ca) and fragment (F). (X 1000)

Figure (8): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F2-group) displaying centric and acentric fragments (F) and centromeric attenuation (Ca). (X 1000)

Fig (9)



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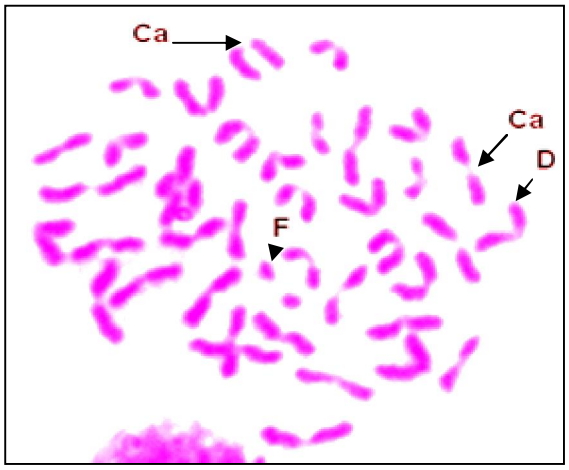

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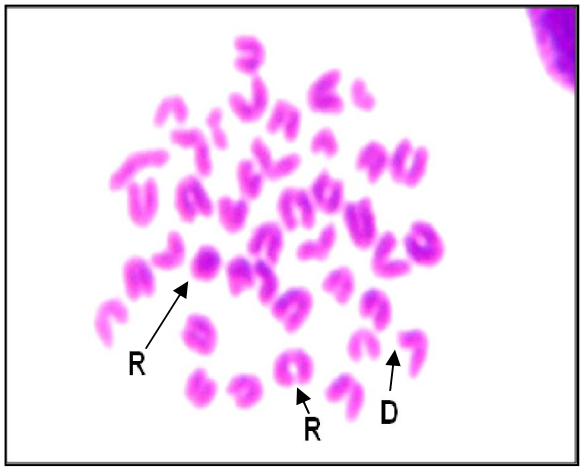

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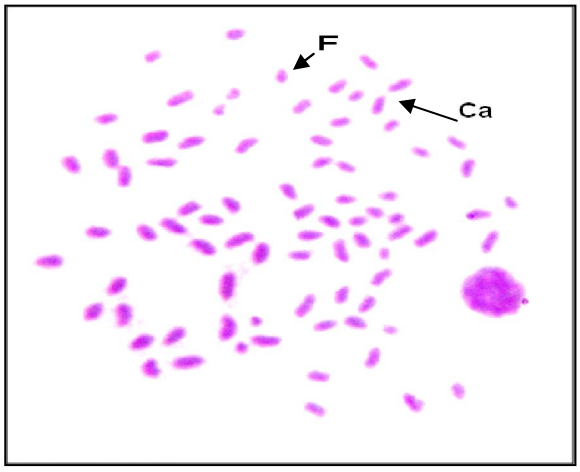


Figure (9): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F4-group) reflecting deletions (D)**.** (X 1000)

Figure (10): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F4-group) showing centromeric attenuation (Ca), deletion (D) and fragment (F). (X 1000)

Figure (11): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F4-group) displaying ring chromosomes (R) and deletion (D). (X 1000)

Figure (12): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F4-group) detecting centric and acentric fragments (F) and multiple chromosomes in separating centromeric attenuation form (Ca). (X 1000)

Fig (13)

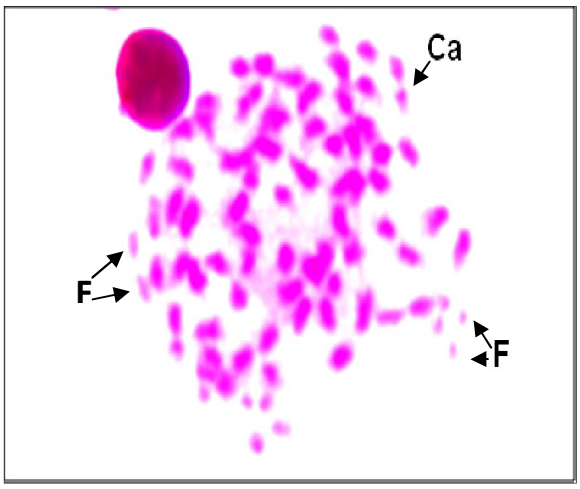
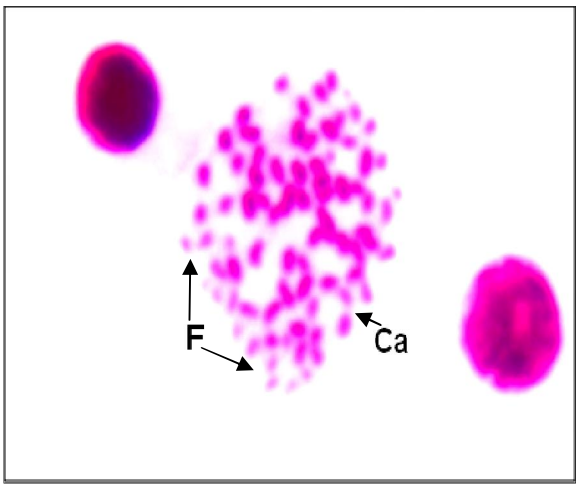


Fig (14)



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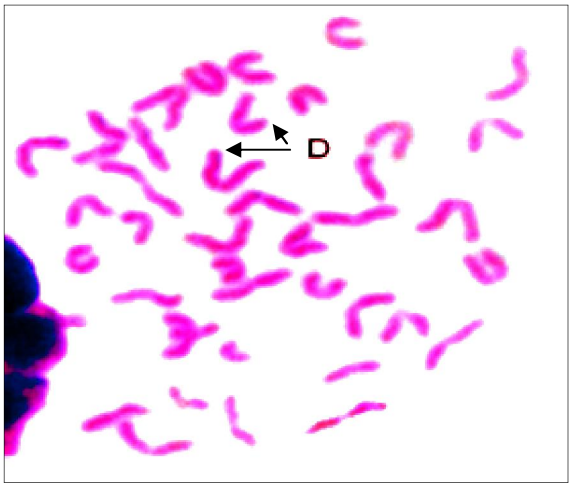

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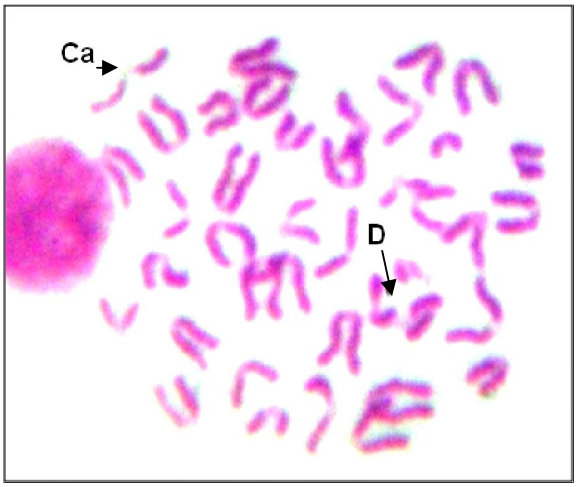


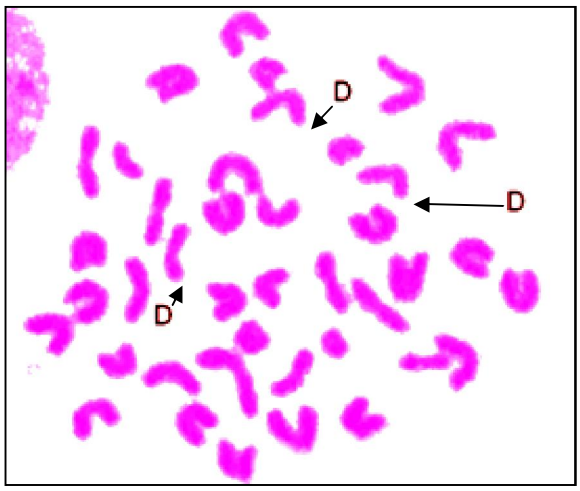
Figure (13): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F4-group) displaying centric and acentric fragments (F) and centromeric attenuation (Ca), taking condensation appearance**.** (X 1000)

Figure (14): Photomicrograph of metaphase chromosomes of 5-FU treated mouse (F4-group) exhibiting centric and acentric fragments (F) and centromeric attenuation (Ca), taking condensation and sticky appearance**.** (X 1000)

Figure (15): Photomicrograph of metaphase chromosomes of 5-FU and Vitamin C treated mouse (FV2-group) reflecting deletions (D). (X 1000)

Figure (16): Photomicrograph of metaphase chromosomes of 5-FU and Vitamin C treated mouse (FV2-group) showing centromeric attenuation (Ca) deletion (D). (X 1000)

Fig (17)



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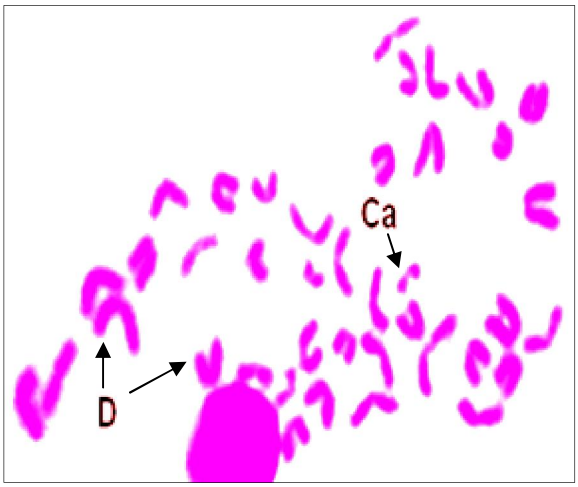

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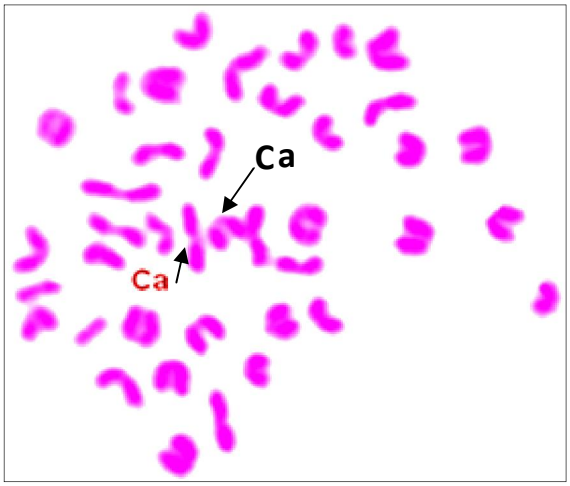

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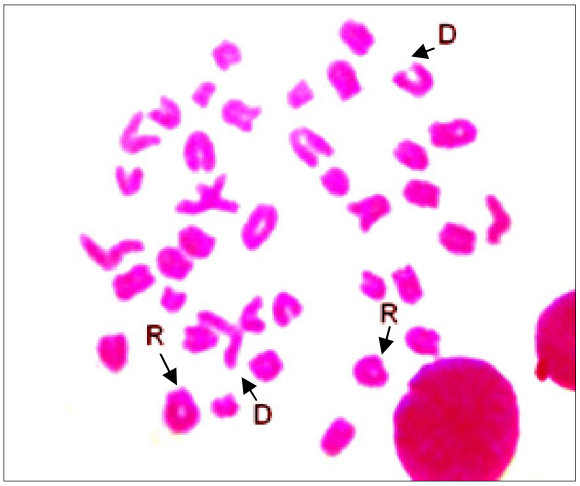

Figure (17): Photomicrograph of metaphase chromosomes of 5-FU and Vitamin C treated mouse (FV4-group) showing deletions (D). (X 1000)

Figure (18): Photomicrograph of metaphase chromosomes of 5-FU and Vitamin C treated mouse (FV4-group) reflecting centromeric attenuation (Ca) and deletions (D). (X 1000)

Figure (19): Photomicrograph of metaphase chromosomes Vitamin C treated mouse (V-group) detecting centromeric attenuation (Ca), and others take a rather normal appearance. (X 1000)

Figure (20): Photomicrograph of metaphase chromosomes Vitamin C treated mouse (V-group) exhibiting deletions (D) and ring chromosome (R), and others appeared in normal shape. (X 1000)

Note: all 5-FU treated groups were received the equivalent therapeutic dose (80 mg/kg b.wt.) in every other days. Whilst, all vitamin C injected groups were given the equivalent therapeutic dose (12 mg/kg b.wt.) daily.

Table 1: Means (±SD) of chromosomal aberrations in 500 metaphases/5 mice per each experimental group and its total mean (± SD) after treatment with 5-FU in concurrent or separately with the protective vitamin C for two and four weeks, and their respective control mice.

Animal groups	Means (±SD) of chromosomal aberrations in 500 metaphases/5 mice per each experimental group				Total Mean (±SD) of chromosomal aberrations
	Deletions	Centromeric attenuation	Fragments	Ring chromosomes	
Control mice (C group)	0.6 ± 0.547	0.6 ± 0.894	0.4 ± 0.547	0.6 ± 0.894	2.2 ± 0.447
5FU-Treated mice (F2 group)	35.4** ± 4.393	26** ± 2.915	13.6** ± 4.505	4.8* ± 2.489	79.8** ±4.494
5FU-Treated mice (F4 group)	2.2* ±1.303	43.2** ±2.387	32.6** ±5.941	2 ±1	80** ±5.338
5FU&Vitamin C-Treated mice (FV2 group)	9.6** ±2.408	6.4** ±2.701	11.2** ±3.271	0 ±0	27.2** ±2.588
5FU&Vitamin C-Treated mice (FV4 group)	2* ±0.707	14.4** ±3.361	25.6** ±3.361	0 ±0	42** ± 5.099
Vitamin C-Treated mice (V group)	1.4 ±0.547	0.4 ± 0.547	0.6 ±0.457	0.8 ± 0.836	3.2 ±0.836

Level of significance: $P > 0.05$ Insignificant. $P < 0.05$ (*) Significant. $P < 0.001$ (**) Highly significant.

Table 2: Mitotic index (MI) in 1000 bone marrow cells per each experimental group and its percentages after treatment with 5-FU in concurrent or separately with vitamin C for two and four weeks, and their respective control mice.

Animal groups	Score of divided cells / total cells (1000)	Percentages of MI
C-group	380	38 %
F2-group	205	20.5 %
F4-group	238	23.8 %
FV2-group	325	32.5 %
FV4-group	300	30 %
V-group	357	35.7 %

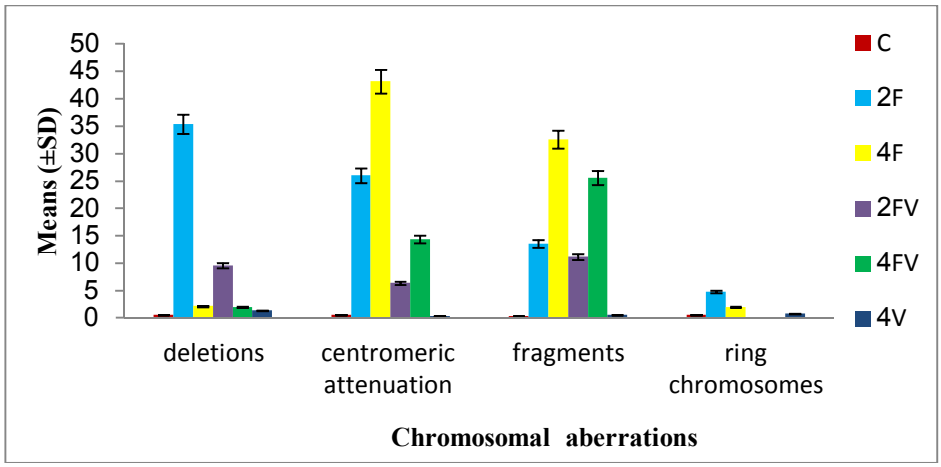

Figure 21: Histogram of means (±SD) of chromosomal aberrations in all five groups and their respective control, as recorded in table 1.

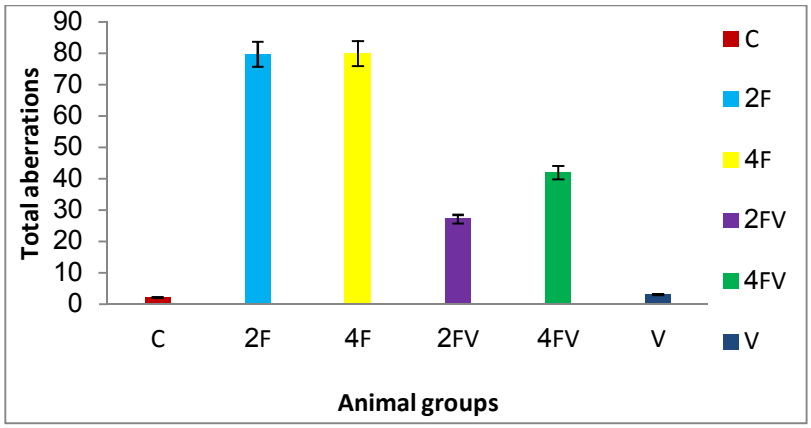

Figure 22: Histogram of means (±SD) of total chromosomal aberrations in all five groups and their respective control, as calculated in table 1.

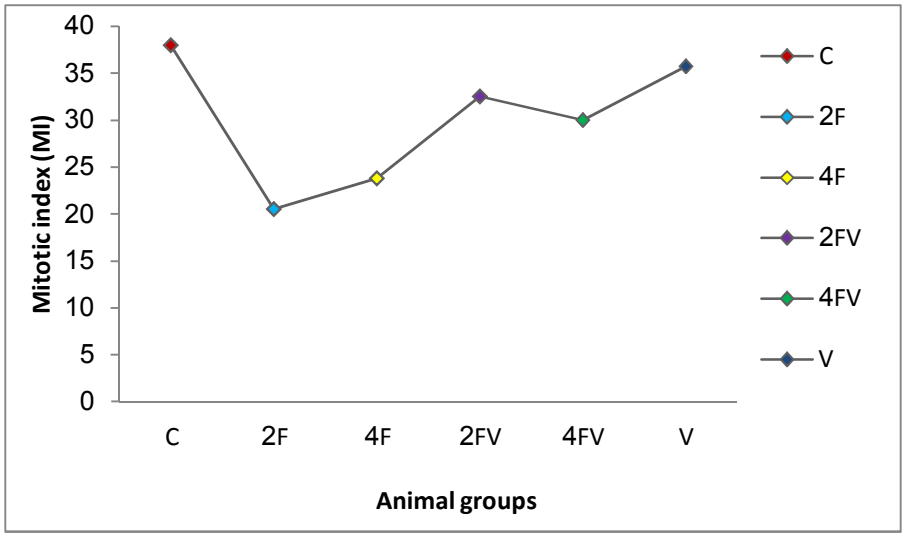

Figure 23: Histogram of percentages of mitotic indices (MI) in all five groups and their respective control, as calculated in table 2.

4. Discussion

The present study involved the following structural chromosome aberrations after treatment with 5-FU:

- **Deletions**

This abnormality indicates the absence or loss of a segment at the end of one chromatid of a chromosome as a deletion, as perceived in **figures (**2-4, 6, 7, 9- 11, 15-18 & 20). From the statistical view, the highest mean value of deletions was observed with the treated group (F2), recording (35.4). Whilst, the lowest frequency of such aberration was detected with the protective group (FV4), manifesting (2), as illustrated in **table (1)** and **figure (21).**

- **Centromeric Attenuation**

This case is marked by the fact that the centromere of a chromosome is inclined to either stretching or splitting. In the former instance, the two chromatids join together by a very thin chromatin thread, as observed in **figures** (2, 4- 8, 10, 12-14, 16, 18 & 19), whereas in the latter (splitting) condition, the two chromatids are completely separated from each other as in **figures (8, 12, 13** & **14)**. The statistical analysis of this aberration in the 5-fluorouracil-treated and protective groups showed that the highest value of centromeric attenuation was observed with the treated group (F4), exhibiting (43.2). Whilst, the lowest once of such abnormality was detected with the protective group (FV2), recording (6.4), as shown in **table (1)** and **figure (21)**.

- **Fragments**

In this aberration, the metaphase spread involves one or more chromosomes in fragmented appearance. However, some chromosomes in this abnormality occurred in centric or acentric type, as

represented in **figures (5, 7, 8, 10, 12-14)**. The highest mean value of this aberration was observed with treated group (F4), recording (32.6). While, the lowest frequency of such abnormality was observed in the protective group of (FV2), manifesting (11.2), as illustrated in **table (1)** and **figure (21)**.

- **Ring Chromosomes:**

The ring chromosome is essentially formed by a break involving both telomeric ends of a chromosome, followed by deletion of the broken acentric fragments in addition an end to end fusion of the remaining two chromosome arms, as demonstrated in **figures (3- 7, 11** & **20).** The highest frequency of ring chromosomes is exhibited with the treated group (F2), recording (4.8). However, the protective groups (FV2) and (FV4) didn't show that aberration, as shown in **table (1)** and **figure (21)**.

In this work, the chromosomes of 5 FU-treated mice, at 80 mg/kg b.wt. for two periods (two; F2 and four weeks; F4) showed conspicuous structural aberrations. The structural abnormalities produced in F2 and F4 groups appeared as deletions s, centromeric attenuation, ring chromosome and fragments (centric and acentric) but in different frequencies. However, **Epstein (1984)** found that Patients receiving cumulative doses of 0.24-1.0 g of 5-FU have shown an increase in numerical and structural chromosomal aberrations in peripheral blood lymphocytes. However, **Rønne** and **Andersen (1978)** suggested that the structural alteration of the chromosomes after 5-fluorouridine (as derivative of 5-FU) treatment was due to a partial inhibition of RNA synthesis in late G_2 phase and interference with posttranscriptional modifications of tRNA and rRNA. Furthermore, the data by **Pullarkat *et al*. (2001)** suggested that genotyping for the thymidylate synthase (*TS*) gene polymorphism may determine the response and toxicity of 5-FU chemotherapy. Whereas, the *TS* gene catalyses the conversion of deoxy-uridylate to deoxy-thymidylate, which is essential for DNA synthesis. So, the individuals homozygous of *TS* gene had 3.6 times higher TS mRNA levels compared to those homozygous for in tumour tissue. In addition to all of the above general discussion concerning the 5FU-induced chromosomal aberrations, in the present material, certain comments are thereafter offered on those structural aberrations.

From the statistical view, in the present work, the highest mean value of deletions was observed with the treated group (F2) exhibiting a statistically highly significant increase (P<0.001) relative to the control group. In this concern, **Sokova** and **Volgareva (1979)** investigated that 5-FU was hardly injured the chromosomes of human tumour cells carrying out chromatid deletions and gaps formed the major part of drug-induced cytogenetic abnormalities about reached 94%. Whilst, the lowest frequency of such aberration was detected at the protective group (FV4) reflecting a statistically highly significant increase (P <0.01) comparable to the control group. Such results were in agreement with **Rosselli *et al*. (1990)** who detected such chromosomal aberrations, in rat peripheral lymphocytes after various time intervals (3, 12, 24 or 48 hrs) with acute treatment of 5-FU (50 mg/kg) causing a remarkable increase (13-fold) in chromosomal damage was observed at the first sampling time. Then within 48 hrs the effect was drastically reduced but persistent (3 times the control level). In **(1982), Swanson *et al.*** designated that chromosomal deficiency or deletion was represented a quantitative change in the genotype involving the loss of genetic material. Thus, it would be speculated that deletions would have deleterious impacts on an organism, depending upon the amount of genetic material and its specific function.

The present study showed that statistical analysis of centromeric attenuation aberration in both 5 FU-treated groups and protective groups carried out the highest value of centromeric attenuation was, which observed as the treated group (F4) exhibiting a statistically highly significant increase (P<0.001) relative to the control group. Whilst, the lowest once of such abnormality was detected with the protective group (FV2) manifesting statistical highly significant increase (P <0.01) comparable to the control group. **Dolara *et al*. (1994**) described the same phenomenon of chromatid separation under the name "nonsynchronous centromeric separation". The authors concluded that the disturbances of the spindle filaments are likely to be the cause of the disruption of the centromeric apparatus during mitosis, which manifested itself as a chromatid separation. In which, the centromeric attenuation (taking splitting form of the centromere without mitosis) may be an early stage of endomitosis, in which case it gives rise to polyploidy **(De Handet *et al*., 1983; Ito** and **Matsumoto, 2010).** In **(2012**), **Matsunaga**, ***et al***. reported that the cohesion alternation of centromeric region was essential for the identification of sister chromatids and for the biorientation of chromosomes until their segregation. Moreover**,** the same authors proposed that RBMX (RNA-binding motif protein encoded on the X chromosome) was a cohesion regulator that maintained the proper cohesion of sister chromatids. Such protein (i. e., RBMX) when depleted from chromosomes caused the loss of cohesin factor from the centromeric regions before anaphase, resulting in premature chromatid separation accompanied by delocalization of the kinetochore proteins. However, the present investigation adopted such hypothesis by **Matsunaga**, ***et al***. **(2012**), whereas the used drug 5-

FU may be affect on the essential cohesion RBMX protein factor at the centromeric regions between the sister chromatids, resulting in the chromosome aberration of centromeric attenuation. Concerning the mechanism action of another breast cancer medicine of "tamoxifen" on the centromeric regions, as generated in a mouse strain was also detected by **Perera *et al*. (2007).** In which, the study illustrated that the role of tamoxifen administration was inactivation of a factor Bub1, which is surveillance for all the chromosomes to be stably attached to spindle microtubules *via* their kinetochores and protecting centromeric cohesion.

The cytogenetic studies by **Berrozpe *et al*. (1990)** on a bladder carcinoma, carried out using short time cultures, showed centromere splitting form mainly affecting chromosomes 22, 13, 14, 21, 15, 20, 12, 7, 17, and 18. The authors suggested that centromere splitting type was an early phenomenon in the karyotypic evolution of aneuploidy in bladder cancer. In a study of chromosome fragility induced under folate and thymidine deficiency conditions, observed a seven- to nine folds increase of the incidence of premature centromere divisions (PCDs) affecting all chromosomes (**Fuster *et al*., 1992**). This early separation of centromere was clearly a culture effect and distinct from PCD and centromere splitting, which implied a defect in the centromere of one or more chromosomes.

The frequency of fragment aberration in this study was markedly time-dependent in bone marrow cells of male mice treated. The statistical analysis of this aberration in both 5-flurouracil-treated groups and protective groups showed that the highest value of fragments was observed in the treated group (F4) reflecting a statistical highly significant increase ($P<0.001$) relative to the control group. The same results were in agreement by **Choudhury *et al*. (2001),** who recorded that the chromatid breaks and fragments in bone marrow cells of mice, by all the three different concentrations (5, 10 and 15 mg/kg) of 5-FU, were detecting statistical significant increase, as an agent of S-phase dependent cytogenetic toxicity. Whilst, the lowest once of such abnormality was detected with the protective group (FV2) manifesting statistical highly significant increase ($P<0.001$) comparable to the control group. In this aspect, **Casati *et al.* (1995)** suggested that this aberration was anomalous chromatin behavior could derived from alteration of the correct chromosome condensation, which associated with increase of micronuclei. Besides, **Alvarez *et al*. (1997)** described this aberration with multifragments in degenerated germ cells structures.

The highest frequency of ring chromosomes in the present study was in exhibited the treated group (F2), recording (4.8). However, the protective groups (FV2) and (FV4) didn't show that aberration. From the statistical view, the highest value of ring chromosome was observed with the treated group (F2), exhibiting (4.8) and reflecting a statistically highly significant increase ($P<0.01$) relative to the control group. Whilst, the lowest once of such abnormality wasn't detected with the protective groups (FV2) and (FV4) and manifesting statistically significant decrease ($P<0.05$) comparable to the control group. According to **Swanson *et al.* (1982),** the experimented individuals possessing ring chromosomes were exhibited certain phenotypic abnormalities. The authors also supposed that the ring shape of the bacteriophage chromosome was due to the complementary redundancy of a single polynucleotide sequence which terminates the chromosome.

Mitotic index (MI) is defined as the ratio (%) of the mitotic-phase-arrested cell number to the total cell number **(Ikeda *et al*., 2000**). Generally, they also added that the MI was reduced by anti-tumor agents, suggesting that they blocked the cell cycle before 2h of the mitotic phase. Thus, the measurement of the G2-index contributes to the screening of putative antitumor agents.

The results of this study recorded that the elevation rate of mitotic index at different periods of administration with vitamin C may affect the target mitotic rate of bone marrow cells induced anticancer drug 5-FU.

The decline rate of mitotic index of 5-FU injected mice was due to its antimitotic activity, so that topical 5-FU is a useful therapy for the treatment of many dermatological disorders characterized by a high mitotic rate, such as skin cancers and benign tumours **(Yen-Moore, 2009; Gauthier *et al*., 2013)**.

The percentages of mitotic indices were decreased in these results in a time-dependent and linear manner at administration with the antimetabolite drug "5-FU". But that was inversely was applied in concurrent or separately with vitamin C when compared with control group. Such result were confirmed with **Azevedo *et al*. (2012)**, who pointed out there was a marked mitotic arrest in 5-FU treated mice was causing a disrupted growth factor signaling in crypt intestinal mucositis of wild-type mice and was ameliorated by apolipoprotein E COG 133 mimetic peptide.

However, the application of vitamin C in both FV2 and FV4 groups may be increased the division rate, as illustrated by mitotic indices of experimental animals were due to its impacts on apoptotic average, as in agreement with **Shionome *et al*. (2013),** interpreted such inhibition of mitotic index of 5-FU treated mice was completed by suppressing of

tumourigenesis induced by aurora A-kinase; which is an essential factor necessary for cell proliferation, and further induces apoptosis.

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