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THE EFFECT OF GINGER TINCTURE AND OLIVE OIL ON THE EXPERIMENTALLY INDUCED BONE MARROW DEPRESSION IN ADULT MALE ALBINO RAT

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ABSTRACT

Background: Cyclophosphamide (CP) is a nitrogen alkylating agent used in various types of cancer chemotherapy, in spite of that CP chemotherapy induces sever bone marrow damage and depression. Many natural compounds derived from plants proved to have a protective effect against the toxic effects of many chemicals, such as ginger plant which is considered as a rich source of antioxidants, while olive oil plays a well established, beneficial role in promoting good health. **Material and method:** Forty adult male albino rats were divided into four equal groups: GI; control group, GII; ginger and olive oil receiving group, GIII; CP injected group, GIV; group receiving both CP with ginger and olive oil. This experiment continued for 15 days. At its end blood samples were collected for complete blood picture and bone marrow samples were taken for histological, immunohistochemical and chromosomal studies. Morphometric and statistical studies were also done. **Results:** CP caused prominent deficiency in the hemopoietic cells within the bone marrow, it decreased the RBCs, WBCs and platelets count in peripheral blood. CP produced depression of dividing bone marrow cells. This was detected by PCNA immunohistochemical study. Also, it produced some chromosomal structural changes. All these changes were improved when CP given in association with ginger tincture and olive oil. **Conclusion:** Administration of ginger tincture and olive oil is playing a role in protection of the body against the harmful effects of some toxic agent as CP.

KEY WORD: cyclphosphamide, ginger, olive oil, bone marrow and chromosomes.

INTRODUCTION

The majority of anticancer (antineoplastic) drugs are especially designed to interfere with DNA synthesis, cellular metabolism and cell division. Due to this mode of action, these drugs are expected to cause mutations and cytogenetic abnormalities. Therefore, anticancer drugs are used as mutagens in most antimutagenic tests.[1]

CP is a nitrogen alkylating agent used in various types of cancer chemotherapy, in spite of that CP chemotherapy induces sever bone marrow damage and depression[2]. The International Agency for Research Centre has identified it as a carcinogen for both animals and humans. CP therapy causes injuries and peroxidative damage to normal tissue and other vital organs in which such side effects are supported by the reactive oxygen species produced by the metabolic activation of CP in the liver by cytochrome P450.[3]

During recent years, considerable efforts have been focused on using antimutagens to modulate the genotoxic effects of the mutagenic antineoplastic drugs [1]. Many natural compounds derived from plants proved to have a protective effect against the toxic effects of many chemicals. Ginger plant is considered a rich source of antioxidants[4]. It has been cultivated for thousands of years as a spice and for medicinal purposes. Several population-based studies showed that people in South East Asian countries have a much lower risk of colon, gastrointestinal, prostate, breast, and other cancers than their Western counterparts [5], and it is thought that constituents of their diet may play significant role in protection. It has been used as a treatment for many diseases such as headache, arthritis, nausea and vomiting.[6, 7]

Olive oil plays a well-established, beneficial role in promoting good health. Phenolic compounds are the main component of the olive oil. Moreover, olive oil is an integral ingredient of a Mediterranean diet. Its nutritional, medical, and cosmetic benefits are widely known and approved. Olive oil is used for combating diseases due to its hypotensive, cardio-protective, antioxidant, antimicrobial, anti-hyperglycemic, anticancer, and anti-inflammatory pharmacological

properties. It could release chronic diseases such as cardiovascular disease, atherosclerosis, and some types of cancers – especially colorectal and breast cancer.[8]

Therefore, the aim of this work is to study the histological, immunohistochemical and cytogentic changes of bone marrow after exposure to CP and the possible protective effect of both ginger tincture and olive oil in adult male albino rat.

MATERIAL AND METHOD

Forty adult male albino rats, weighing 200g±10g, were used in this experiment. They were housed in ventilated cages in the animal house of histology department, Faculty of medicine for Girls, Al-Azhar University, Cairo, Egypt. Animals were given food (normal rat chow) and free water access. Animals were kept for one week before the beginning of the experiment for acclimatization.

The animals were randomly divided into four equal groups; N=10. Group I: control group. Group II: ginger and olive oil group, in this group the animals received daily oral dose of both ginger tincture and olive oil. Olive oil and fresh ginger were purchased from market. They were given to the animals by gastric tube. The dose of olive oil was 1ml/100 gm body weight[9] while the ginger tincture was 250 mg/kg body weight[7]. The ginger tincture was prepared daily for each chosen rat by adding 50 mg of fresh minced ginger to 2 ml cold water, this mixture was prepared at afternoon, left in refrigerator till the morning of the next day, where it is filtered and given to the animal. Group III: CP group, the animals received CP by intraperitoneal route of administration. The dose of CP was 40mg/Kg body weight alternatively for 15 days. CP was dissolved in distilled water (200mg/ 20ml)[10]. The CP was purchased from market. Group IV: CP and ginger olive oil group, in this group the animals were given both CP, ginger tincture and olive oil by the previously prescribed doses and route of administration.

The experiment was continued for two weeks, after this duration half the number of rats from each group was anesthetized by ether inhalation. Then, the blood samples were collected from retro-orbital venous plexus for complete blood picture which was done in Al-Azhar University Center for Virsus Research and Studies. Bone marrow of both femora from each rat were collected and fixed in Bouin's solution[11, 12]. Then, processed for paraffin blocks formation, serial sections of 5µ thickness were cut and stained with H&E for routine histological examination of bone marrow structure[13]. The slides were examined under the light microscope and the images were taken with microscope connected to digital camera.

For immunohistochemical study, avidin- biotin peroxidase technique for proliferating cell nuclear antigen (PCNA)[14] using PCNA antibodies (Dako-Denmark, diluted 1:100). PCNA antibodies served as a reliable marker of proliferation[15].

Cytogenetic study was done on the remaining half of rats from each group. This half of rats was injected colchicine via intraperitoneal route 2 hours prior to sacrifice. The colchicine is an antimitotic drug. Bone marrow from all groups of animals were collected in freshly prepared hypotonic solution of potassium chloride (KCl), the cell suspension was incubated for 20 min. at 37˚c then centrifuged, the supernatant was discarded, then the cells were resuspended in cold fixative of methanol/acetic acid (3:1). The centrifugation and fixation were repeated twice at an interval of 30 min., finally the cells were resuspended in fixative and were spread by dropping on slides. The slides were flame dried and stained with 10% buffered Giemsa stain.[16]

Morphometric and statistical studies were done for the following: i) The mean number of PCNA positive cells/ high power field was measured in five fields per slide. Five slides were used for each group[17]. ii) The number of chromosomal aberrations was counted in 50 metaphase spread in each group under 1000x (oil immersion lens) microscopic magnification[18]. Statistical analysis was done for the previous parameters using the (t) test for data evaluation and for comparison between the experimental groups. Data was expressed as means ± Standard Deviation (SD). P value ≤ 0.05 was considered statistically significant.

RESULTS

Histological results

Light microscopic examination of H&E stained sections of the control group (GI) showed that the bone marrow consisted of two main components; a meshwork of vascular sinus with interstices packed with hemopoietic cells. In between these components there were few adipocytes distributed throughout the field which might appear as a signet ring. The bone marrow sinuses were lined by flat endothelial cells with their flat basophilic nuclei, RBCs could be recognized within the lumen. The island of hemopoetic cells was composed of blood cells in various stages of maturation, the most prominent and huge cell within these cells was megakaryocyte which was recognized by its large irregular multilobular nucleus usually devoid of nuclei **(Fig. 1).**

The olive oil and ginger group (GII): showed similar bone marrow histological features as those of the control group **(Fig. 2).**

Examination of CP group (G III) in H&E stained sections showed that the bone marrow was formed of interstices of less condensed hemopoietic cells, even some areas appeared to contain very few cells easily to be counted. These cells were replaced by pale homogenous acidophilic areas, some of these areas contained small rounded or oval vacuoles resembling the fat cells even some of these vacuoles appeared as a small

signet ring. Adipocytes were abundant and commonly seen throughout the field while megakaryocytes were less prominent **(Fig. 3).**

Examination of CP, olive oil & ginger (G IV) group showed many interstices of bone marrow packed with hemopoietic cells, few spots that contained few hemopoitic cells, adipocytes were less prominent than those of the previous groups while megakaryocytes were more prominent **(Fig. 4).**

Immunohistochemical results

In control group (G I) and olive oil & ginger group (G II) most of the bone marrow cells showed dark brown cytoplasmic granules, which means PCNA Immuno-positive reaction **(Fig. 5,6),** while fewer PCNA positive cells were detected in G III **(Fig. 7).** In G IV more numerous Immuno-positive cells were detected if compared to the previous group **(Fig. 8).**

Cytogenetic results

Chromosomal examination of the control group in metaphase stage, showed that each chromosome was formed of two chromatids, connected by centromere. According to the position of centromere the chromosomes were classified into metacentric, submetacentric, acrocentric and telocentric **(Fig. 9).** Examination of metaphase chromosomes in olive oil & ginger group showed that the chromosomal structure was similar to the control group **(Fig. 10).** Examination of the metaphase spread chromosomes of the CP group showed chromosomal aberration in the form of fragment, break, deletion, ring chromosome and end to end fusion **(Fig. 11, 12, 13, 14).** The group treated with olive oil & ginger with CP showed that most of metaphase spread was similar to the control, few chromosomal aberrations could be detected in the form of deletion **(Fig. 15)**

Serological results

The blood picture in the control and olive oil & ginger groups showed no statistical significant difference, while CP group showed that the RBCs, WBCs and platelets counts were significantly decreased than the control. The G IV showed significant increase in the RBCs, WBCs and platelets counts but still lower than the control group **(Table 1).**

Morphometric and statistical results

As regard the statistical study concerning PCNA immunoreactions, there was no statistical significant difference between G I and II. Significant decrease in the mean number of positive PCNA cells was detected in CP group (G III) if compared with G I. In G IV, the mean number of positive PCNA cells was significantly increased than the CP group **(Table 2).**

The chromosomal anomalies were counted in 50 metaphase spread for each group and the results were compared to the control. The chromosomal aberrations showed no statistical significant difference between G I and II, while CP group (G III) showed a significant increase of the abnormal chromosomes if compared with the control group. In G IV, the chromosomal aberrations were significantly decreased than the CP group but still higher than the control group **(Table 2).**

Table (1): Mean count of RBCs, total leucocytes and platelets/L in different experimental groups

variable / Studied groups	RBCs count u/L Mean±SD N=10	Total leucocytic count u/L Mean±SD N=10	Platelets count u/L Mean±SD N=10
Control group (GI)	$7.34x10^6$ ±0.27	8000±0.59	$583x10^3$ ±1.21
Olive oil & ginger group (GII)	$7.12x10^6$ ±0.23 [a]	7988±0.57 [a]	$562x10^3$ ±1.3 [a]
CP group (GIII)	$5.3x10^6$ ±0.19* [a]	1600±0.22* [a]	$332x10^3$ ±0.41* [a]
CP with olive oil & ginger treated group (GIV)	$6.5x10^6$ ±0.28* [b]	4800±0.31* [b]	$463x10^3$ ±0.32* [b]

*$P \leq 0.05$ = statistically significant.
[a] compared with control group
[b] compared with CP group

Table (2): Mean number of chromosomal aberrations/50 metaphase spread and mean number of PCNA positive cells in different experimental groups.

variable / Studied groups	Mean number of chromosomal aberrations/ 100 metaphase spread±SD N=10	Mean number of PCNA positive cells±SD N=10
Control group (GI)	0.25±0.31	67±3.21
Olive oil & ginger group (GII)	0.22±0.23 [a]	64±2.92 [a]
CP group (GIII)	0.7±0.64* [a]	41±1.99* [a]
CP with olive oil & ginger treated group (GIV)	0.33±0.21* [b]	55±2.54* [b]

*$P \leq 0.05$ = statistically significant.
[a] compared with control group
[b] compared with CP group

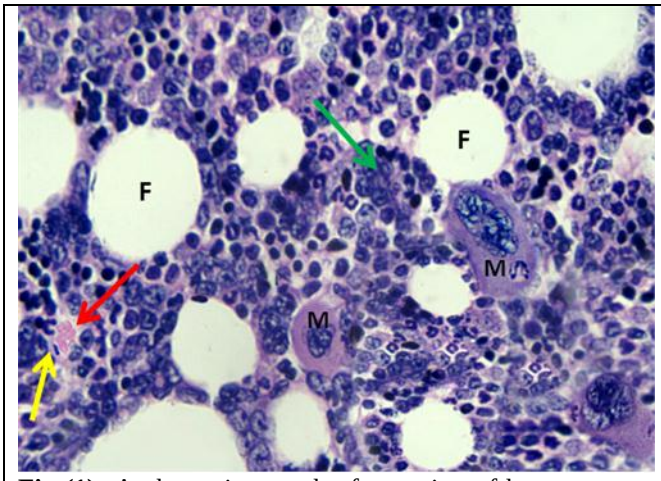

Fig (1): A photomicrograph of a section of bone marrow of an adult male albino rat (G I) showing the bone marrow is composed of: interstices packed with blood cells (green arrow), blood sinusoid (red arrow) lined with endothelial cell recognized by its flat nucleus (yellow arrow), RBCs can be seen in its lumen. Notice, adipocytes (F) and megakaryocytes (M) **[H&E x600].**

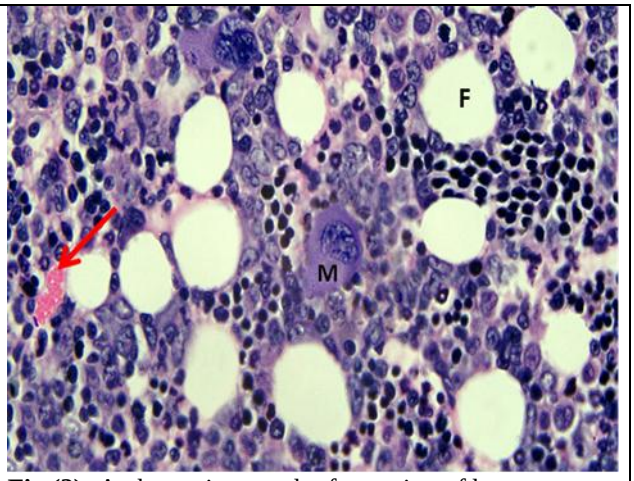

Fig (2): A photomicrograph of a section of bone marrow of an adult male albino rat (G II) showing the bone marrow structure is similar to the control group GI. Notice, blood sinusoid (red arrow), adipocytes (F) and megakaryocytes (M) **[H&E x600].**

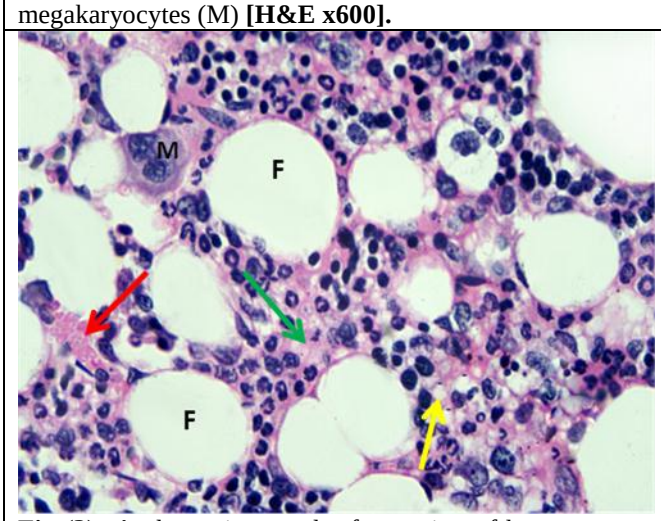

Fig (3): A photomicrograph of a section of bone marrow of an adult male albino rat (G III) showing few hemopoitic cells within the interstices which replaced by homogenous acidophilic materials (green arrow). Small aggregation of acidophilic vacuoles can be seen, some of them takes the signet ring appearance (yellow arrow). More numerous adipocytes (F) and less numerous megakaryocytes (M) can be observed. Notice, blood sinusoid (red arrow) lined with flat endothelial cell and RBCs can be seen with its lumen **[H&E x600].**

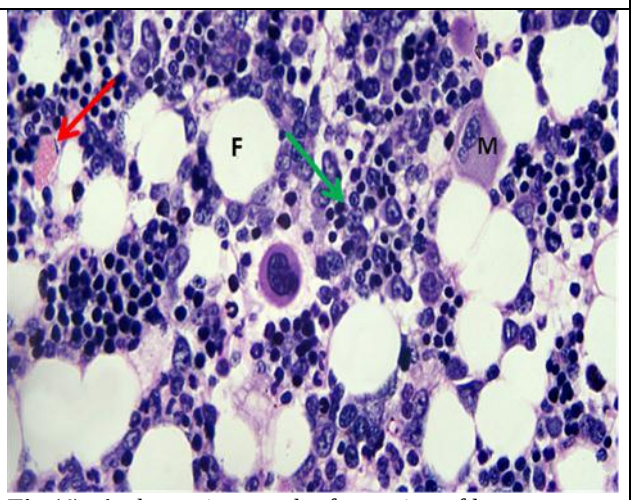

Fig (4): A photomicrograph of a section of bone marrow of an adult male albino rat (G IV) showing more interstices packed with hemopoitic cells (green arrow), fat cells (F) are less abundant and megakaryocytes (M) are more prominent. Notice, blood sinusoid (red arrow) lined with flat endothelial cell and RBCs can be seen with its lumen **[H&E x600].**

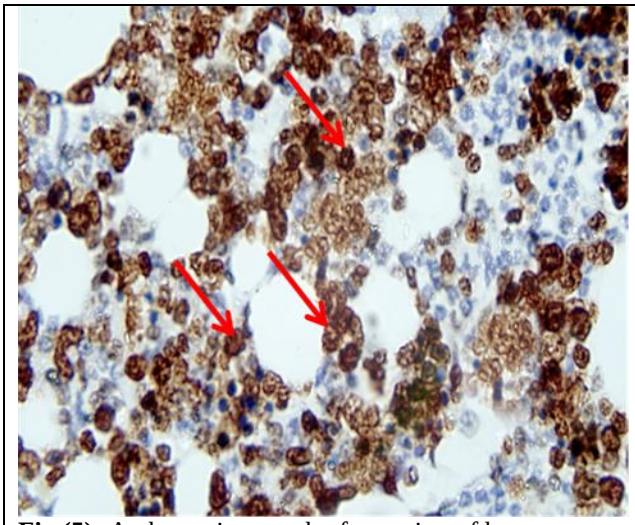

Fig (5): A photomicrograph of a section of bone marrow of an adult male albino rat (G I) showing the bone marrow field is full of strongly positive cells for PCNA (arrows) **[Avidin biotin peroxidase & H x600]**

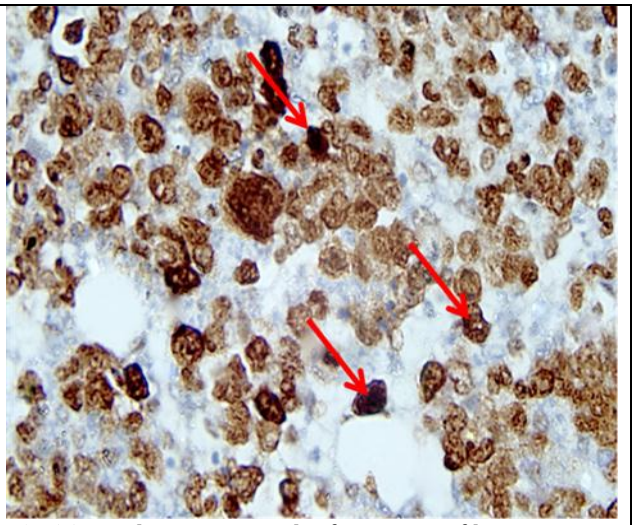

Fig (6): A photomicrograph of a section of bone marrow of an adult male albino rat (G II) showing the bone marrow field is full of strongly positive cells for PCNA (arrows) **[Avidin biotin peroxidase & H x600]**

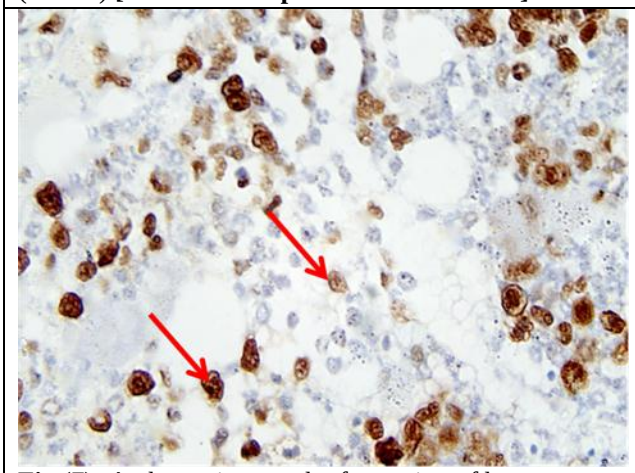

Fig (7): A photomicrograph of a section of bone marrow of an adult male albino rat (G III) showing apparent decrease in PCNA positive reaction in bone marrow cells as compared with that in control group (GI) (arrows) **[Avidin biotin peroxidase & H x600]**

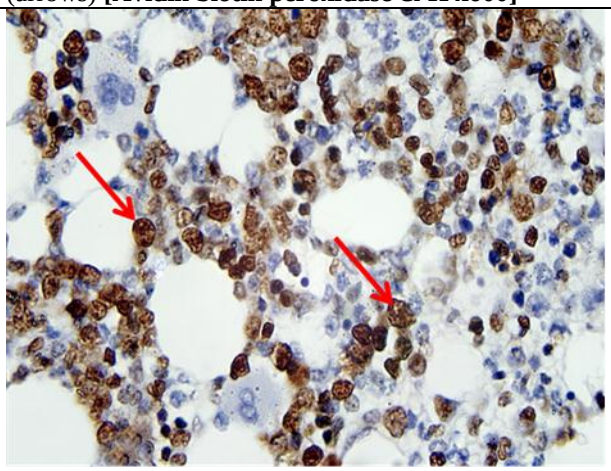

Fig (8): A photomicrograph of a section of bone marrow of an adult male albino rat (G IV) showing apparent increase in PCNA positive cells if compared to the previous group (GIII) (arrows) **[Avidin biotin peroxidase & H x600]**

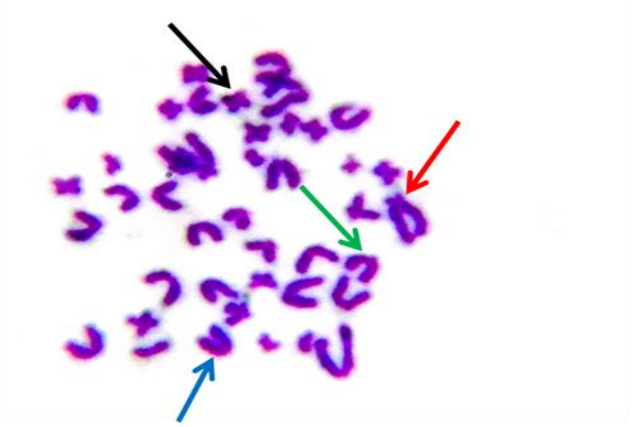

Fig (9): A photomicrograph of metaphase spread of chromosome in bone marrow cells of an adult male albino rat (G I) showing metacentric chromosome (black arrow), submetacentric chromosome (red arrow), telocentric chromosome (blue arrow) and acrocentric chromosome (green arrow) **[Giemsa x1000]**

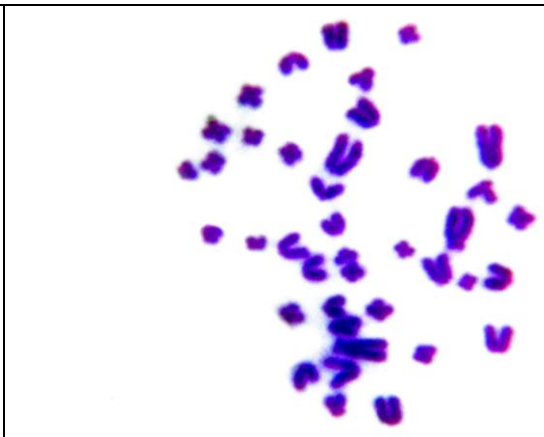

Fig (10): A photomicrograph of metaphase spread of chromosome in bone marrow cells of an adult male albino rat (G II) showing the same chromosomal structure as the control group (GI) **[Giemsa x1000]**

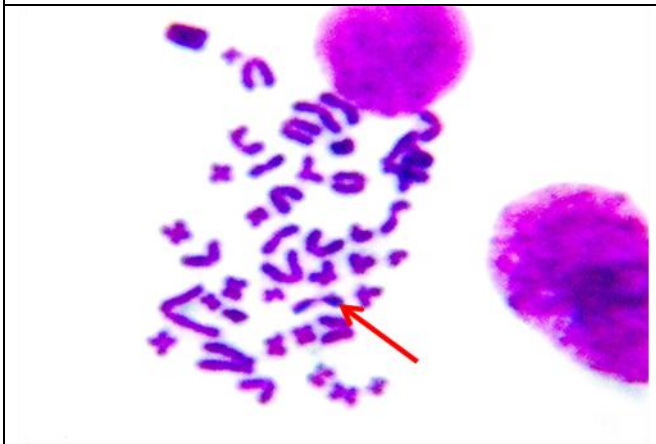

Fig (11): A photomicrograph of metaphase spread of chromosome in bone marrow cells of an adult male albino rat (G III) showing chromosomal break (arrow) **[Giemsa x1000]**

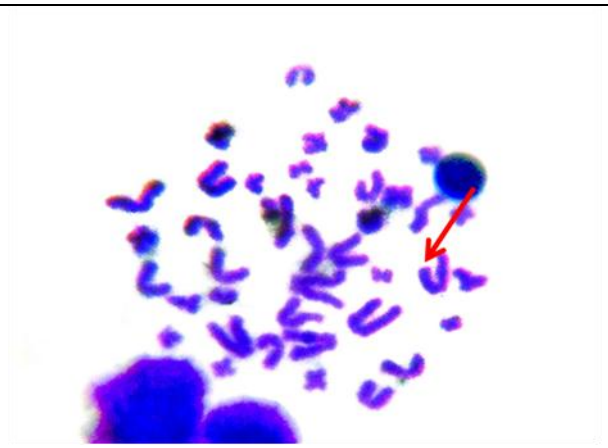

Fig (12): A photomicrograph of metaphase spread of chromosome in bone marrow cells of an adult male albino rat (G III) showing chromosomal deletion (arrow) **[Giemsa x1000]**

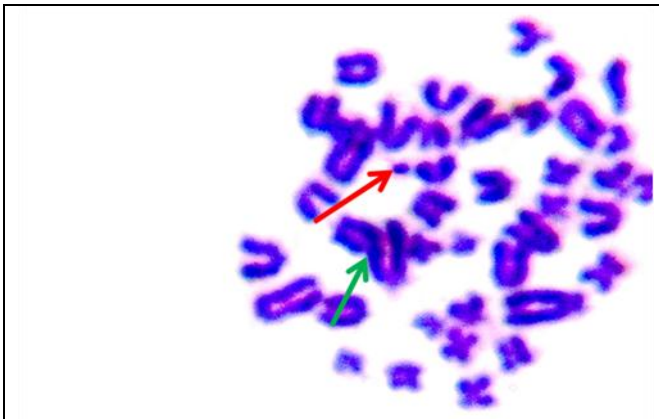

Fig (13): A photomicrograph of metaphase spread of chromosome in bone marrow cells of an adult male albino rat (G III) showing end to end fusion of two chromosome (green arrow) and chromosomal fragment (red arrow) **[Giemsa x1000]**

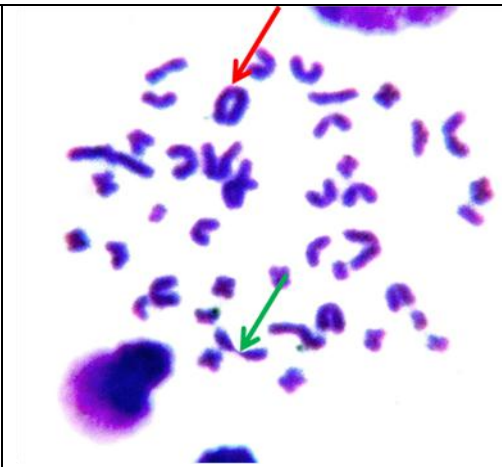

Fig (14): A photomicrograph of metaphase spread of chromosome in bone marrow cells of an adult male albino rat (G III) showing ring chromosome (red arrow) and chromosomal break (green arrow) **[Giemsa x1000]**

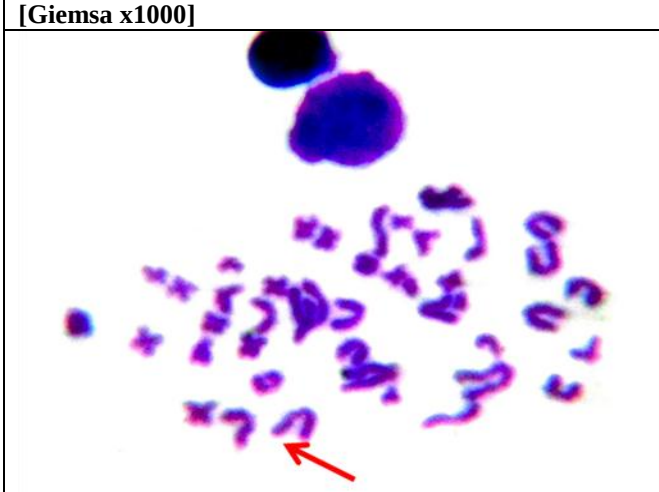

Fig (15): A photomicrograph of metaphase spread of chromosome in bone marrow cells of an adult male albino rat (G IV) showing chromosomal deletion (arrow) **[Giemsa x1000]**

DISCUSSION

Currently, nutrition therapy, in addition to dietary support, can exert therapeutic effects without side effects that accompany classical pharmacotherapy.[19]

Examination of H & E stained bone marrow sections of control and ginger & olive oil groups were more or less histologically similar. This was explained and confirmed by the safety of these two plants as the olive oil was described to have many beneficial effects and it can be considered as functional food having excellent value[20], while ginger is generally considered a safe herbal medicine of insignificant side effects[1].

Examination of bone marrow sections in CP group showed deficiency of haemopoietic cells including megakaryocytes. These changes were represented in the peripheral blood by statistically significant decrease of platelets which are derived from megakaryocytes, and significant decrease of WBCs &RBCs which are derived from other haemopoietic cells [21]. Abundant fat cells and vacuoles were also detected.

These findings were described by[2] who mentioned that the CP chemotherapy causes sever reduction of bone marrow cellularity which was accompanied by an increase in marrow adipose tissue even adipocytes showed expansion in their sizes. Even[5] added that CP causes depression in leucocytes count and low hemoglobin level.[22] explained that the haemopoietic tissue is a one of the rapidly proliferating tissues in which DNA synthesis is intense. Anticancer drugs may induce sever dysplasia and morphological changes in bone marrow and in peripheral blood.

The presence of vacuoles or acidophilic masses instead of haemopoietic cells in the present study, might be explained by that the CP can bind with DNA causing chromosome breaks, micronucleus formation and cell death[23]. Thus the died cells might appear as vacuoles or acidophilic reminents . At the same time the previous data explained the elevated number of abnormal chromosomes in this group of animals (CP group) by the binding of DNA with CP. Also[3, 24, 25] stated that the CP causes DNA damage, genotoxicity and chromosomal damage and aberrations in bone marrow cells.

Adipocytes were more common in histological sections of CP group. The CP suppresses bone marrow activity[2], when bone marrow becomes less active, it is progressively dominated by fat cells [26].

Proliferating cell nuclear antigen (PCNA), is an essential regulator of the cell cycle. It has been shown that PCNA serves as a co- factor for DNA polymerase delta in S-phase and is involved in DNA damage repair during DNA synthesis. The pattern of PCNA expression makes it a useful tool to study cell proliferation. It starts to accumulate in G1 phase of the cell cycle, reaches the highest level during the S phase and decreases during G2/M phase. PCNA may serve as a reliable marker of proliferation processes[15].

In the CP group, the proliferating cells of bone marrow which detected by PCNA immunoreactions was statistically decreased if compared to the control group. This may be explained by the opinion of some authors who stated that the CP disturbs DNA synthesis and cell division [10]. They added that, it is well known that the cancer chemotherapy act by killing cells that divide rapidly (cancer cells). This means, it is also harmful to normal cells that divide rapidly i.e. cells in bone marrow, digestive tract and hair follicle. The previous data may explain why the proliferating cells detected by PCNA immunohisto chemical technique were fewer in CP group than in other groups.

Medicinal plants and their derivatives have been used as an alternative to synthetic medicine in many countries. Medicinal plants play an important role in two sided approach: 1- plant derived compounds are complex in nature which are difficult to synthesize in the laboratory and are helpful in the prevention of onset of cancer by its antioxidant activity and stimulation of the immune system. 2- plant derived compounds are used for prevention and decreasing side effects of conventional cancer treatments[3].

In group IV or group receiving CP with ginger & olive oil, there was prominent amelioration at all levels, in the histological structure of bone marrow, in number of proliferating cells and the number of peripheral blood cell count, even the abnormal chromosomes showed statistical significant decrease in their mean number.

The CP produces its toxicity on tissues by over production of reactive oxygen species (ROS) that cause oxidative stress[10]. Some researchers[3] explained that CP under metabolic activation by cytochrome p450 in liver produces metabolic products such as acrolein (ROS) that cross-links DNA and decreases the antioxidant activity. The antioxidant systems exist in human, can remove ROS. When the ROS level increases and the activation of antioxidant systems decreases, the person is in oxidation stress condition[8].

Ginger contains active phenolic compounds Such as gingerol, paradol and shogoal that have antioxidant properties. Ginger possesses antioxidant and anti-inflammatory properties [1]. This antioxidant activity of ginger makes it able to scavenging the ROS and helps in tissue restoration. On the other hand, the beneficial effects of olive oil can be attributed to the antioxidant property of its phenolic compounds [20].

The statistically significant decrease in number of abnormal chromosomes in group IV was in agreement with some authors[27] who mentioned that when animals were treated with CP showed cytogenetic damage. However, a significant decrease was observed in the percentage of chromosomal aberrations when animals given CP with ginger extract. Other studies reveal that ginger exerts antimutagenic action against carsinogens in vivo and in vitro. Gingerol proved to be effective in reducing genotoxic damage[1]. They added ginger is an antimutagenic agent and having the ability to protect DNA damage. In addition, a number of researchers have proved that the anti-tumor properties of olive oil would prevent DNA damage[28].

For all the previously mentioned information, the combination of both ginger & olive oil acquired the ability to ameliorate the proliferating cells and decreased number of abnormal chromosomes in CP treated rats.

CONCLUSION

The combination of ginger and olive oil exerts a potent protective effect against cyclophosphamide induced toxicity in rat bone marrow, which might be possibly due to the scavenging of free radicals during oxidative stress conditions.

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