Evaluation of radio protective effects of wheat germ oil in male rats

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ABSTRACT: Wheat germ oil posses various biological properties as an anticancer and antioxidant agent. Present study was undertaken to evaluate the radio protective ability of wheat germ (WG) oil against whole body irradiation rat. Wheat germ oil was given to rats by oral injection in a concentration of 1 ml/kg and 3 ml/kg body weight/dose for 3 successive days, last dose administered 24 h pre-irradiation exposure with an acute single dose level of 2 Gry delivered at a dose rate of 0.564 Gry/ min at the time of experiment. With regard to cellular system, the results clearly indicated that pre-treatment with 3ml oil is more potent than 1ml and there are no significant differences between control group and groups that received oil only at either 1ml or 3ml in comparison to the control. Prior administration of WG oil to rats, significantly countered radiation induced biochemical disorder (liver enzymes and kidney function analysis, as well as, cholesterol level in the serum) and DNA damage (evaluated by DNA fragmentation assay and chromosomal aberration in bone marrow) in a dose dependent manner maximally at a concentration of 3 ml/kg. The results clearly indicated that wheat germ oil has significant potential to protect cellular system from radiation induced damage and ability to scavenge free radicals might be playing an important role in its radio protective manifestation without any toxicity

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1. INTRODUCTION

Radiotherapy is one of the most common therapies for treating human cancers. Several studies have indicated that irradiation induces reactive oxygen species (ROS), which play an important role in irradiation damage of the cell. Scientific and technological advancements have further increased the radiation burden in humans, because exposure to low levels of radiation has become common during medical diagnostic procedures, space or air travel, cosmic radiation and through the use of certain electronic gadgets. Other sources of radiation exposure include radon in houses, contamination from weapons testing sites, nuclear accidents and radiotherapy. The amounts of ionizing radiation that can be given to treat tumors are often limited due to the surrounding normal tissues and organs in the vicinity of the tumor that could be also exposed to the radiation causing damage, (Kunwar *et al.*, 2010). Ionizing radiation may cause cancer, death, and loss of neural function in humans and animals. It also induces mutation, chromosomal aberrations and apoptosis in cells, Nair, *et al (*2001); Jagetia and Reddy (2005).

Among the various physical/chemical agents, radiation is an important source in the generation of oxygen-derived free radicals and excited states. In actively metabolizing cells, there is considerable water apart from the target macromolecules of DNA, proteins, lipids and so on. The exposure of biological systems to radiation results in a radiolytic cleavage of water, giving rise to OH^- and H^+. However, ionizing radiation can break chemical bonds and cause ionization of biologically important macromolecules such as nucleic acids, membrane lipids and proteins, (Lett 1992 and Daniniak and Tann 1995; Kamat *et al.*, 2000). Due to the presence of polyunsaturated fatty acids, membranes are highly susceptible to oxidative damage induced by reactive oxygen species (ROS) generated during radiation, (Rice-Evans and Burdon (1993). Hence, compounds that are capable of protecting cellular membranes against ionizing radiation in particular, and free radicals in general, will have potential benefits as radio-protectors, antioxidants and anti-mutagens, Stavric (1994). Antioxidant systems have a fundamental role in defending organisms against irradiation-induced oxidative stress.

Antioxidants are molecules that can prevent or reduce the extent of oxidative destruction of bio-molecules when present in small concentrations compared with the bio-molecules they are supposed to protect, Halliwell, (1990).

The essential oils and extracts of many plant species have become popular in recent years, and attempts to characterize their bioactive principles have gained momentum in many pharmaceutical and food processing applications, (Cowan, 1999). Plants (fruits, vegetables, medicinal herbs) contain a wide variety of free radical-scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites, that are rich in antioxidant activity, (Larson, 1988; Shahidi & Naczk, 1995; Cotelle *et al.*, 1996; Velioglu, et al., 1998; Zheng & Wang, 2001; Cai, *et al.*, 2003). Natural radio protectors are found in plant materials such as oil seed and wheat germ oil, (Singh *et al.*, 2009).

Wheat is an important source of vitamins, minerals, dietary fibre and phyto-chemicals. The oil is a rich source of toco-pherols and toco-trienols. The germ is the most nutritious portion of the wheat and it makes up about 2.5 % of the weight. During the milling process the germ is separated from the bran and starch. Wheat germ is a rich source of B complex vitamins, with wheat germ oil being the richest source of tocopherols. These nutrients and phytochemicals may have significant implications in chemoprevention, (Jensen *et al.*, 2004; Lui, 2007). This oil is a source of easily assailable vitamin E which acts as inhibitor of oxidation processes in body tissues. It protects cells against the effects of free radicals, which are potentially damaging by products of the body's metabolism. Free radicals can cause cell damage that may contribute to the development of cancer, (Traber *et al.*, 1999).

Recent studies have shown that a fibre rich diet reduces or causes a delay in fat digestion, impedes the absorption of cholesterol and fat in the intestine, reduces cholesterol synthesis by volatile fatty acids produced during fermentation and alters lipoprotein metabolism, (Cara *et al.*, 1992). A study conducted by Boateng *et al* (2007) concluded that dietary fat, depending on the source, quantity, fatty acid composition may have implications in the incidence of colon cancer. Wheat germ oil not only prevents autoxidation of unsaturated fatty acids but also generates DNA protective properties, (Gelmeza *et al.*,2009) .Hence it would be beneficial to determine the radio protective effects of wheat germ oil at 1 and 3 ml/kg which represents a normal and a high fat diet composition in the irradiated rats.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. Chemicals:

Wheat germ oil was purchased from El-Captain Company (CAP PHARMA), 6th October City, Egypt. All other chemicals were of analytical grade.

2.1.2. Irradiation:

Source of irradiation used for Cobalt-60 gamma cell 3500. Whole-body Gamma-irradiation was performed at Middle Eastern Regional Radioisotopes Centre for The Arab Countries, Dokki, Cairo, Egypt. Animals were irradiated at an acute single dose level of 2 Gry delivered at a dose rate of 0.564 Gry/ min at the time of experiment.

2.1.3. Animal and Housing

Before commencing the work we obtained permission from the Institutional Animal Ethics Committee of NRC. Sixty Wister albino rats weighing 120 - 150 g (average age, 12 weeks) were used in this study. The animals were housed in stainless steel wire cages at 5 rates per cage under standard laboratory conditions. They were fed standard laboratory chow and water before the experiment for one week.

2.1.4. Wheat germ oil

In the present work wheat germ oil was given to rats by oral gavage in a concentration of 1 ml/kg and 3 ml/kg body weight/dose for 3 successive days as previously described based on the preliminary study, last dose administered 24 h pre-irradiation exposure.

2.2. Methods:

2.2.1. Experimental Design:

At the beginning of the experiment, rats were divided into six groups (10 rats / group).

The 1st one was the untreated control group.

The 2nd group was exposed whole body to a single dose of γ-irradiation (2 Gry).

The 3rd and 5th groups were treated with wheat germ oil alone by oral administration at 1 and 3 ml/kg respectively for three consecutive days.

The 4th and 6th groups were treated with 1 and 3 ml/kg respectively for three consecutive days pre-exposed to γ-irradiation.

All rats were left one hour after last dose of the treatment before exposure to γ-irradiation.

2.2.2. Analytical procedures:

After 24 hours post-irradiation, rats were dissected under light anaesthesia and blood sample were collected by heart puncture using sterile syringes. Blood samples were incubated at 37°C then centrifuged to collect sera for biochemical analysis. Liver was dissected out and bone marrow was obtained for cytogenetic analysis (DNA fragmentation and chromosome aberrations).

2.2.3. Biochemical analysis:

For liver enzymes, serum ALT and AST were determined by kinetic method according to German Society for Clinical Chemistry (1970), Total serum cholesterol was determined by enzymatic colorimetric method according to Richmond (1973). To determine kidney function, serum creatinine was estimated by kinetic kits according to Henry (1974). While, blood urea determined by enzymatic and colorimetric method according to Patton and Crouch (1977).

2.2.4. DNA Fragmentation Assay:

Animals within different treatment groups were sacrificed 24h after last treatment and samples were collected. The method of DNA fragmentation assay was carried out according to Perandones *et al.* (1993). Rat liver was mechanically dissociated in hypotonic lysis buffer. The cell lysate was centrifuged at 11.000 rpm for 15 min. then the supernatant containing small DNA fragments was separated immediately, half the supernatant was used for gel-electrophoresis. The other half, as well as the pellet containing large pieces of DNA were used for the colorimetric determination Diphenylamine (DPA) assay.

2.2.5. Chromosomal aberrations:

In somatic cells, bone-marrow metaphases were prepared according to Yosida and Amano (1965). Briefly, bone marrow from the femur was aspirated, washed in saline, treated hypotonically (0.565% KCl), fixed in 3:1 methanol: acetic acid, spread on clean slides. Slides were stained with 7% Giemsa stain in phosphate buffer (pH 6.8). 100 well spread metaphases per animal were analyzed for chromosome aberrations. The types of aberrations in bone-marrow cells included breaks, deletions, fragments, centric fusions, centromeric attenuations, etc.

2.2.6. Statistical analysis:

Data were analyzed using One-way analysis of variance (ANOVA). The results obtained were expressed as means ± standard error of the mean. Differences were considered significant at $P<0.05$.

3. RESULTS

The data of the present work showed the serum level of liver enzymes (AST and ALT), serum cholesterol and kidney function (serum creatinine and urea) of rats exposed to a single dose of γ-radiation (2 Gry) and rats pre-treated with 1 and 3 ml wheat germ oil. Serum level of liver function and kidney function were described in Table (1).

While serum cholesterol level illustrated in Fig 1. Serum AST revealed significant increases ($P< 0.05$) in rats exposed to γ-radiation than other treatments. WG oil decreased the elevation of serum AST activity particularly when rats orally received with 3 ml WG oil before exposure to γ-radiation. No significant differences among group III and V when compared with healthy control group. On other side, serum ALT level showed significant elevation in groups II, IV and VI as compared with control. Pre-treatment with 3ml oil is more potent than 1ml to decrease the elevation level of ALT as a result of irradiation exposure and this means that 3 ml oil is more ameliorate the harmful effects of irradiation. No significant differences were observed between untreated control rats and rats either treated with 1ml or 3ml.

There were decreases in the level of S. creatinine as a result of administration of oil before irradiation exposure in groups IV and VI but the decrease was pronounced at 3ml pre-treatment when compared with irradiated group.

However, a significant decrease in s. urea in groups IV and VI in comparison with group II, where as no significant change was obtained when compare each other .Serum cholesterol level declared significant elevation ($P< 0.05$) in the irradiated group and other groups which pre-treated with oil then exposed to irradiation II, IV and VI, when compared with control (I). However, the decrease was slightly more in the group pre-treated with 3ml oil but without significant difference with 1ml pre-treatment. There were no significant differences between control group and groups that received oil only at either 1ml or 3ml in compared to the control.

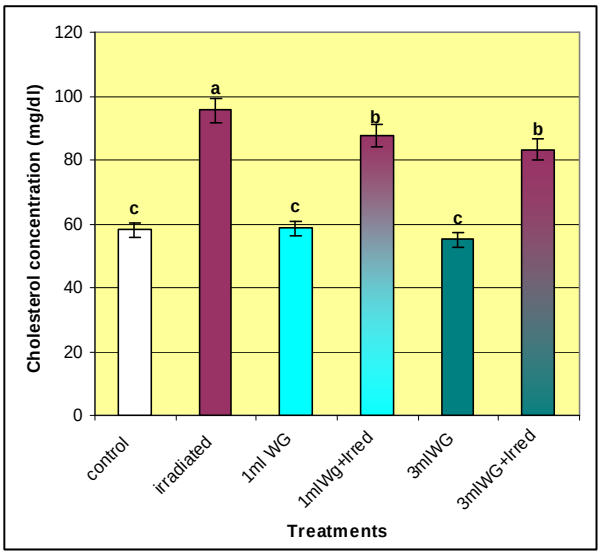


Figure 1: Histogram showing the effects of wheat germ oil (WG) on the concentration of serum cholesterol in the different rat groups.

Table 1: Effects of wheat germ oil (WG) on liver enzymes and kidney function of different rat groups

Parameters	Control I	Irradiation (IR) II	1ml WG III	1ml WG & IR IV	3ml WG V	3ml WG &IR VI
S. AST U/L	271.38 ± 6.01 d	555.2 ± 3.89 a	251.18 ± 4.7 e	489.0 ± 1.09 b	248.32 ± 4.8 e	414.6 ± 5.87 c
S. ALT U/L	54.96 ± 2.1 d	189.39 ± 0.86 a	56.2 ± 1.02 d	150.45 ± 4.4 b	56.0 ± 1.79 d	124.33 ± 1.63 c
S. creatinine mg/dl	0.6 ± 0.005 d	0.88 ± 0.014 a	0.59 ± 0.008 d	0.83 ± 0.009 b	0.59 ± 0.007 d	0.71 ± 0.007 c
S. urea mg/dl	27.84 ± 0.42 c	36.38 ± 1.34 a	27.22 ± 0.46 c	33.1 ± 1.13 b	25.9 ± 0.48 c	32.0 ± 0.57 b

Data are means of five replicates ± standard error. Means in the same raw have the same letter are not significantly different at 0.05

DNA Fragmentation Results:

The results presented in table (2) indicated that the percentage of DNA fragmentation in liver was increased significantly in irradiated animals compared to the control. Animals treated with WG alone at 1 and 3 ml/kg showed insignificant increase in the percentage of DNA fragmentation. On the other hand, animals treated with WG oil at both concentration and exposed to irradiation showed a significant decreased in the percentage of DNA fragmentation towards the control values although these treatments did not normalize it. Moreover, this improvement was pronounced in the liver of animals treated with WG at 3 ml/kg prior to irradiation. DNA fragmentation in response to irradiated animals was also detected by gel electrophoresis as DNA ladder representing a series of fragments that is multiples of 180–200 bp (Fig 2)

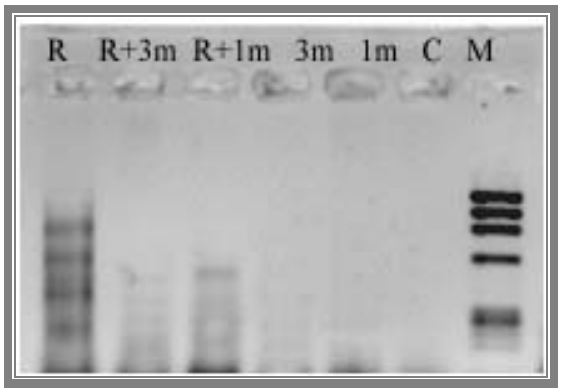


Figure 2: Agarose gel electrophoresis showing the effects of wheat germ oil (WG) on percentage of DNA fragmentation on liver of irradiated rats Lane M: DNA molecular weight marker. Lane C: control group; Lane 1m: wheat germ oil at 1 ml, Lane 3 ml: wheat germ oil *at 3 ml; Lane R+1m: Irradiated rats then received WG at 1ml; Lane R+3ml: Irradiated rats then received WG at 3ml; Lane R: irradiated rats.*

Table 2: Effects of wheat germ oil (WG) on percentage of DNA fragmentation on liver of different rat groups

Treatment	DNA Fragmentation %	The change %
Control (I)	7.9	--
Irradiation (II)	32.0	+ 24.1
1 ml / kg WG (III)	9.6	+ 1.7
1ml/kg WG + Irradiation (IV)	17.4	+ 9.5
3 ml / kg WG (V)	10.0	+ 2.1
3ml/kg WG + Irradiation (VI)	14.95	+ 7.05

Chromosome aberrations:

The frequencies of different types of structural (breaks, deletion, fragment and centromeric attenuation) and numerical chromosomal aberration (polyploidy and aneuploidy) for different treatment were presented in Table (3) and Fig (4). Total chromosomal aberrations showed a significant increase ($P< 0.05$) in γ-irradiated group compared to the control group. While animals treated with WG oil at 1 or 3 ml/kg body weight induced insignificant increased in the frequency of chromosome aberrations (3.8 ± 0.66 and 4.6 ± 0.68 respectively) when compared with that of the control groups (3.0 ± 0.32). A corresponding increase was found in all the individual aberrations in irradiated animals, however, the number of chromatid break and centromeric attenuation was found to be the most types of aberrations which significantly increased in all irradiated animals. Treatment with WG oil at either 1 ml or 3 ml before irradiation resulted in very significant decrease in the percent of aberrant cells (19.2 and 14.4 % respectively, (Fig 3A) and in the total number of aberrations (9.6 ± 0.86 and 7.2 ± 0.68 respectively) compared to the irradiated-group (19.3 ± 1.56) Table (3). The pre-treatment with oil at both doses inhibited the frequency of chromosomal aberrations in bone marrow by 59.5 % at 1ml /kg while it reached to 74.2% at 3ml/kg, Fig (3B). The present data showed that administration of wheat germ oil ameliorate and improve the harmful effects of irradiation particularly when rats pre-treated with 3ml of the oil.

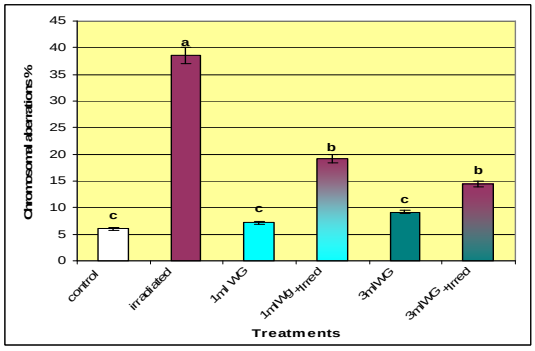
DISCUSSION

Ionizing radiation produces harmful effects on the organisms and due to wide spread use of radiation in diagnosis therapy, industry, so pharmacological intervention could be most potent strategy to protect human or ameliorates the deleterious effect of ionizing radiation, (Jagetia 2007). Ionizing radiations induce significant elevation in the physiological and metabolic processes, as well as, disorders in blood biochemical parameters, El-Masry and Saad (2005) and causing chain reaction of oxidation (Ammar, 2009). The degree of cellular damage is variable among different organs depending on the organ ratio sensitivity, so one approach to prevent such injury is by supplementation or administration with natural potent antioxidant as natural radio-protector (Zhou *et al.*, 2001).

Radiation exerted significant ($P< 0.05$) elevation in the liver enzymes, cholesterol and kidney function. So current investigation in wheat germ oil was applied in view of minimizing the toxicity of ionizing radiation, Ammar (2009) and this agreed with the result of the present work that declared

significant elevation in liver enzymes, cholesterol, creatinine and blood urea as a result of γ-radiation exposure where as this elevation were alleviated when treated with wheat germ oil before γ-radiation exposure. In the present study, treatment with wheat germ oil without exposure to radiation revealed non significant changes in the investigated parameters indicating its safe in use.

(A)



(B)

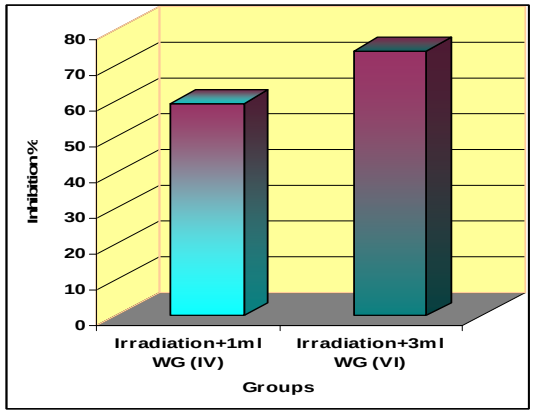


Figure 3: Histogram showing the effects of wheat germ oil (WG) on the: A) percentage of chromosome aberrations and B) The inhibition percent in the irradiated rats

Sisodia *et al.*, (2007) and Kunwar *et al.*, (2010) and reported that ionizing radiation induce augmentation in the levels of serum AST and ALT that were significantly ameliorated by pre-treated with natural radio-protector which agreed with the present work.

Gamma irradiation caused significant increase in the levels of serum kidney and liver peroxidation but pre-treated with natural radio-protector were significantly decreases the level of serum kidney and liver function when compared to the irradiated group, Adaramoye *et al.,(* 2010) and these agrees with the results of the present work but on other side, Adaramoye *et al.*, (2010) reported that these were no significant difference (P>0.05) in the level of serum urea of irradiated and pre-treated animals when compared to the control. Increase in serum urea was due to increase in glutamate de-hydrogenase enzyme as a result of irradiation and this may increase carbamoyl phosphate synthetase activity leading to increase in urea concentration, Ramadan *et al.*, (2001). Administration of WG oil before exposure to γ-irradiation reduced the level of serum urea when compared to the irradiated rats.

Natural radio-protector protected albino rats from the adverse effects of whole body irradiation, Adaramoye *et al.*, (2010). Gamma irradiation induced significant elevation in the level of serum cholesterol on the first day post-irradiation but administration with natural radio-protector before irradiation exposure restored the normal level, Ramadan *et al.*, (2002) and El-missiry *et al.*, (2007) and these were agreed with the present data. The administration of wheat germ oil before irradiation results in an increase in membrane permeability and fluidity causing decreased triglycerides and cholesterol, Yousri *et al.*, (1991).

Exposure of cells to ionizing radiation during the G0 or G1 phases of the cell cycle causes chromosomal aberrations (CAs) as breaks, dicentrics, acentrics, fragments, rings and translocations. These CAs are used as biomarkers of radio-sensitivity or radiation damage after medical, accidental and occupational exposure. Besides, some of these CAs may be strongly linked with different cancer types (Atanasova *et al.*, 2004; Hande *et al.*, 2005; Varella-Garcia *et al.*, 2007). Additionally, radiation is a well-known inducer of free radicals caused to chromosomal damages. In the present study exposure to γ-irradiation (2 Gry) induced chromosomal aberration in bone marrow and DNA fragmentation in liver by 38.6 % and 32.0 % respectively. The use of certain materials may help to decreasing of the genotoxicity created by radiation and may inhibit mutagenesis and carcinogenesis, De Flora *et al.*, (2007).

The results of our present study demonstrate the radio protective effect of wheat germ oil on radiation induced chromosomal aberrations and DNA fragmentation; wheat germ oil itself does not have any marked effect neither on the bone marrow chromosomes nor the DNA fragmentation percent. Wheat germ oil is a source of easily assimilable vitamin E which represented by 89.3 % and acts as inhibitor of oxidation processes in body tissues, Abd El-Azeim *et al.*, (2005). Vitamin E has antioxidant and free radical scavenging activities, which suggests that this vitamin may modulate oxidative DNA damage in mammalian cells, (Odin, 1997, Paranich et al., 2000 and Jacobs et al., 2001). This activity could reduce the incidence of chromosomal aberrations caused by free radicals generation, Abd El-Azeim *et al.*, (2005).

WG oil at both doses (1 ml and 3 ml) inhibited radiation-induced chromosomal aberrations by about 59.5 and 74.2 % respectively, indicating that WG oil has anti-mutagenic effect. In the same manner, Abd El-Azeim *et al.*, (2005) reported that, both olive and wheat germ oils inhibited CP-induced chromosomal aberrations by about 76.8 % indicating that they have anti-clastogenic and anti-mutagenic effects. Similar trends were seen in studies by Field *et al.*, (2008) where rats feed WG oil at 7 and 14 % significantly reduced the number of aberrant crypt foci which were good predictors of tumor outcome, Ishizuka *et al.*, (2003). Wheat germ oil, a significant source of phyto-chemicals such as vitamin E may have played a beneficial role in reducing chromosomal aberration and DNA fragmentation induced by radiation exposure.

Table 3: Effects of wheat germ oil (WG) on the Frequencies of chromosome aberrations induced by exposure to 2.0 Gry of gamma rays in bone marrow of rats

Groups	Total Aberrations	Structural aberrations					Numerical aberrations	
		Ch. break	deletion	Fragment	Centromeric attenuation	Dicentric	Polyploidy	Aneuploidy
Control I	3.0 ± 0.32 ^{d}	0 ^{c}	0.6 ± 0.25 ^{c}	0.6±0.25 ^{b}	0.8±0.37 ^{c}	0 ^{b}	0.6±0.24 ^{b}	0.4 ± 0.25 ^{c}
Irradiation (IR) II	19.3 ± 1.56 ^{a}	2.8 ± 0.37 ^{a}	3.2 ± 0.2 ^{a}	2.4 ± 0.24 ^{a}	6.8 ± 0.37 ^{a}	1.1 ± 0.24 ^{a}	1.8 ± 0.37 ^{a}	1.2 ± 0.37 ^{a}
1 ml/kg WG III	3.8 ± 0.66 ^{d}	0 ^{c}	0.2 ± 0.2 ^{c}	0.4 ± 0.25 ^{b}	1.7 ± 0.2 ^{c}	0 ^{b}	0.8 ± 0.37 ^{b}	0.5 ± 0.24 ^{c}
1ml WG+IR IV	9.6 ± 0.86 ^{b}	1.8 ± 0.2 ^{b}	1.2 ± 0.37 ^{b}	1.0 ± 0.20 ^{b}	3.0 ± 0.32 ^{b}	0.4 ± 0.2 ^{b}	1.0 ± 0.25 ^{b}	1.2 ± 0.25 ^{a}
3ml/kg WG V	4.6 ± 0.68 ^{d}	0.4 ± 0.25 ^{c}	0.4 ± 0.25 ^{c}	0.8 ± 0.20 ^{b}	1.4 ± 0.25 ^{c}	0 ^{b}	0.8 ± 0.2 ^{b}	0.8 ± 0.37 ^{bc}
3ml WG+IR VI	7.2 ± 0.68 ^{c}	1.4 ± 0.4 ^{b}	1.4 ± 0.32 ^{b}	1.2 ± 0.32 ^{b}	1.8 ± 0.37 ^{c}	0 ^{b}	1.0 ± 0.32 ^{b}	0.4 ± 0.37 ^{c}

Ch break = Chromatid break, WG = wheat germ oil

Data are means of five replicates ± standard error. Means in the same column have the same letter are not significantly different at 0.05

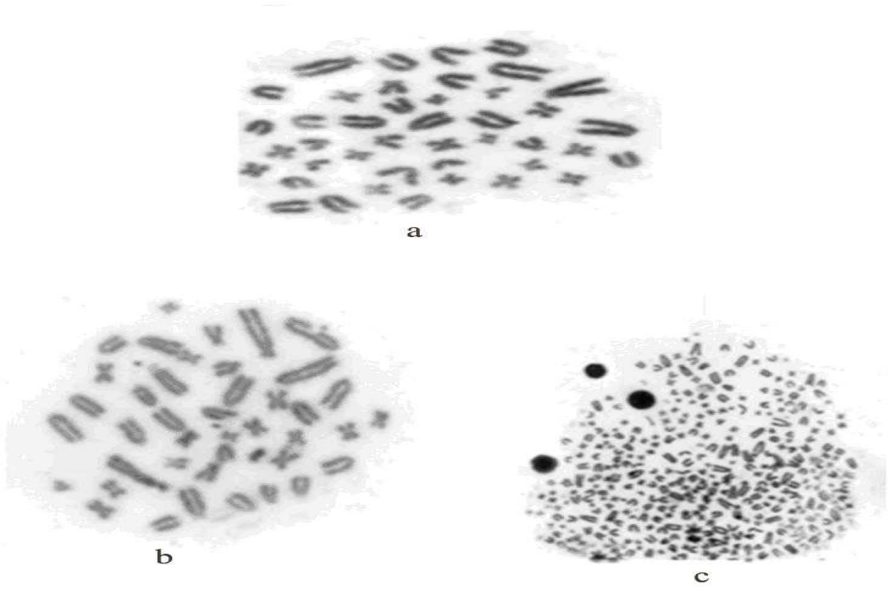


Fig 4: Photo showing: (a) normal spread metaphase, (b) spread metaphase with chromatid break, fragment and deletion, (c) spread metaphase with polyploidy chromosomes

In conclusion, the treatment of rats with wheat germ oil either at 1ml or 3ml /kg body wt prior to whole body γ-irradiation seems to exert protective effects. The mechanism by which wheat germ oil mediated its effects may be due to inhibiting free radical liberating or scavenging these free radicals causing alleviate the damage induced in liver and kidney cells. Results from such studies should provide information of substantial value for making a better decision concerning the future use of wheat germ oil as a preventive measure to reduce injury from exposure to ionizing radiation.

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