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Assessment of the Possible Protective Role of Olive oil on Iron sulfate Induced Genotoxicity Using Chromosomal Aberration Assay and RAPD-PCR in Male Mice

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ABSTRACT

This study was conducted to evaluate the genotoxic effects of iron sulfate on mice through the assessment of chromosomal aberration, mitotic activity and DNA fragmentation by RAPD-PCR, as well as screening for the possible ameliorating effect of extra-virgin olive oil (EVOO) against iron sulfate genotoxicity. Sixty-four swiss albino male mice (Mus musculus), weighing from 25 to 35 gm were used in the study. Forty mice were used in the acute study for 24 and 48 hrs and twenty-four were used for chronic treatment (six weeks). In both acute studies, the mice were divided into five groups, the first group acts as a control without any treatment. The second group received 75 µl EVOO on a diet for one day before samples collection. The third group received 61.2 mg/kg b.wt iron in a diet which represents 1/5 of its oral LD50, the fourth group received the same previous doses of EVOO and iron sulfate at the same time. The fifth group received EVOO as a pre-treatment for one week then received iron sulfate with the previous dose for 24 hrs. Four similar experimental groups were also used for chronic study with 15.3 mg/kg b.wt iron sulfate in the diet which represents 1/20 of its oral LD50 and the same dose of EVOO as a co-treatment. Iron sulfate in mice ration was able to produce structural chromosomal aberration in acute treatment and structural and numerical in chronic treatment, lowering mitotic activity and induced DNA fragmentation (RAPD fragments) at both treatments. EVOO as a co-treatment or pre-treatment leads to decrease in chromosomal aberration especially chromatid breaks, chromatid deletion and fragments in acute treatment. In general, EVOO as a co-treatment showed a significant role in lowering Iron sulfate genotoxicity in acute treatments.

1. INTRODUCTION

Heavy metals are naturally occurring elements. They can release into the environment and produced adverse health effects among human and animals. Human activities lead to increase the heavy metal concentrations in the environment. The industry, agriculture, and solid waste disposal increase the heavy metals contents in groundwater, soil, air, fruits, vegetables, fish and animals (Batayneh, 2010).

Normal cellular functions need a trace amount of some heavy metals that act as an active site for a number of enzymes involved in the oxidation-reduction reaction. On the other hand, the heavy metals are toxic to the cells once their levels exceed their low physiological values, leading to disorders in vital functions (Warchałowska-Śliwa et al., 2005 and Chen and Lippmann, 2009). Heavy metals are the genotoxic, that able to induce structural and numerical

chromosomal aberrations. Also, it can induce DNA fragmentation and micronucleus (Obe et al., 2002).

Iron (Fe) is an essential trace element used by living organisms, which is incorporated into the heme complex, mediate redox reactions, and oxygen transport proteins as hemoglobin in red blood cells and myoglobin in muscle cells. It is also required for the immune system response and energy production (Ganong, 2007). Excess iron causes a serious damage to the liver that is the main storage site of iron in the body, by forming hydroxyl-radical mediated oxidative stress. Iron overload mediated disorders like liver fibrosis, cirrhosis, inflammation, diabetes, impaired cardiac function, arthritis and cancer (Goodman and Gilman, 2006; Barton, 2007 and Chaudhuri et al., 2016).

Several studies have been directed to determine the potential induction of chromosomal aberrations, reticulocytes micronuclei, DNA break by iron and iron containing compounds (Lima et al., 2011 and Parveen et al., 2014).

Nowadays, natural dietary antioxidants have been given attention as possible therapeutic and protective agents against free radicals as a tool to combat oxidative stress (Khalil et al., 2013). Extra-virgin olive oil (EVOO), the major dietary fat component in the Mediterranean diet, has shown anti-inflammatory, immunomodulatory, anti-proliferative, and anti-apoptotic effects (Sánchez-Fidalgo et al., 2013). EVOO has an ameliorative effect against some heavy metals toxicity like chromium and aluminum due to it contain phenolic compounds which act as antioxidants against reactive oxygen species through preventing first chain initiation by scavenging initiating radicals, metal chelating, decreasing localized oxygen concentration, and decomposing peroxides (Owen et al., 2000; Ghorbel et al., 2015 and Saber et al., 2015).

The random amplified polymorphic DNA (RAPD) is a technique using short, an arbitrary primer which is capable of binding at a wide and variable area of the genome. It has been used to determine DNA adduct/ breaks, structural genomic DNA, and mutations affected by toxic chemicals as heavy metals, pesticides and polycyclic aromatic hydrocarbons (Atienzar and Jha, 2006).

Therefore, the aim of the present work was to study the acute and chronic genotoxic effects of iron sulfate on mice by using the assay of chromosomal aberration and mitotic activity as well as the assessment of DNA fragmentation by RAPD-PCR and to screen for the possible ameliorating effect of EVOO against genotoxicity of iron sulfate.

2. MATERIAL AND METHODS

2.1. Animals

Sixty four Swiss albino male mice (*Mus musculus*), weighing from 25 to 35 gm were used in the study. The mice were obtained from a private farm of experimental animals at the Faculty of Agriculture- Al-Shatby- Alexandria. They were housed in plastic cages for one week prior to experiment for acclimatization. The mice received standard balanced ration and water *ad.libitum*. The hygienic condition was kept constant throughout the experimental period. This study was approved by a local ethics committee of Alexandria University.

2.2. Experimental design

2.2.1. Acute Experiment (24 hrs and 48 hrs)

Forty mice were used in this experiment; they were divided into five groups. Each group contained eight mice. The first group acts as a control without any treatment. The second group received 75 µl EVOO according to (Khalil et al., 2013) in diet for one day before samples collection. The third group received 61.2 mg/kg b.wt iron sulfate (El-Gomhouria Company, Egypt) in diet which is 1/5 of oral LD50 (LD50 = 306 mg/kg b.wt) for mice according to (Budavari et al., 1996). The fourth group received the same previous doses of EVOO and iron sulfate at the same time in the diet for 24 hrs. The fifth group received EVOO with the previous dose for one week then gave iron sulfate with previous dose for 24 hrs.

2.2.2. Chronic treatment (six weeks)

Twenty four mice were used in the experiment; they were divided into four groups, each group contained six mice. The first group acts as a control without any treatment. The second group received 75 µl EVOO according to **(Khalil *et al.*, 2013)** in diet for six weeks before samples collection. The third group received 15.3 mg/kg b.wt iron sulfate in the diet which

is 1/20 of oral LD50 (LD50 = 306 mg/kg b.wt) for mice according to **(Budavari *et al.*, 1996)**. The fourth group received the same previous dose of EVOO and iron sulfate at the same time in the diet for six weeks.

2.3. Cytogenetic analysis

2.3.1. Chromosomal aberration assay and Mitotic Index (M.I)

Sample collection from the experimental groups after 24 hrs, 48 hrs and 6 weeks of treatment. Mice were injected intraperitoneal (I.P) with an aqueous solution of colchicine at dose 4 mg/kg b.wt. Then were sacrificed 2 hrs after colchicine injection. Both femurs were removed quickly, flushed bone marrow out and prepared according to **(Giri *et al.*, 1986)**. Slides were stained by 4% Giemsa stain for 5-10 min, then washed by dH_2O and air-dried, and the quality of staining was checked. Thirty, well spread metaphase plate/mouse were randomly selected for screening the structural and numerical chromosomal aberration. The stained slide are used also for scoring of mitotic index (M.I) for control, acute (24 and 48 hrs), chronic (6 weeks) treated groups. 1500 cells were scored for each animals and M.I were calculated according to the equation of Brusick (1980).

2.4. DNA Extraction and RAPD-PCR

Extraction of DNA was done from blood by using G-spin™ Total DNA Extraction Kit (Intron Biotechnology, Korea), following the manufacturer's protocol. RAPD PCR was done using 13 single, short, random primer sequences **(**Table 1). The reaction was performed in a mixture of 25 µl containing: 3.5 µl genomic DNA, 5 µl of Primer (10 Pmol), 12.5 µl master mix, and 4 µl dH_2O. The reaction was performed in Technee, TC-3000, USA-thermal cycler and the PCR program was carried out by initial denaturation at 95°C for 5 min followed by 40 cycles of 94 °C for 1 min (denaturation), annealing at different temperature to each primer listed at Table (1) for 1 min and at 72 °C for 1 min (extension), then at 72 °C for 10 min (final extension). The RAPD-PCR fragments were separated on 2% ethidium bromide-stained agarose gel.

Table (1): Primer sequences used for RAPD-PCR.

Primer	Primer sequence (5'–3')	Annealing temperature
115	TTCCGCGGC	43
137	GGTCTCTCCC	43
CH1	GAATGCGACG	34
CH2	ATGACGTTGG	34
CH3	CTGAGGAGTG	34
CH5	ACCGGGAACG	34
OPA 04	AATCGGGCTG	43
OPA 05	AGGGGTTCTTG	34
OPA 07	GAAACGGGTG	34
OPA 08	GTGACGTAGG	34
OPA 10	GTGATCGCAG	34
OPA 12	TCGGCGATAG	34
OPA 13	CAGCACCCAC	34

3. RESULTS

3.1. Chromosomal aberrations

Iron sulfate alone or with EVOO induced different types of chromosomal abnormalities, they include, fragments, chromatid deletion, chromatid break, centric fusion, ring chromosomes, stickiness, and polyploidy (Fig. 1, Table 2, 3). Numerical chromosomal aberration was observed in bone marrow of chronic treatment only **(**Table 4). However, structural aberrations observed in bone marrow of both acute and chronic treatments were found to be nearly similar.

The different types of aberrations of control and treated groups for 24 hrs are presented in table (2). The group treated with iron sulfate showed the higher significant means in all types of aberration including fragments, chromatid deletion, chromatid break, ring chromosome and stickiness (figures 1A- E) compared to the control groups. The most dominant type of chromosomal aberration of the iron-treated groups was fragment. The chromatid breaks was the lowest frequency for the treated groups.

Iron sulfate significantly increased the number of fragments and ring chromosome compared to the other groups. EVOO pre-treatment and co-treatment with iron sulfate showed a significantly decreased fragments and ring chromosome.

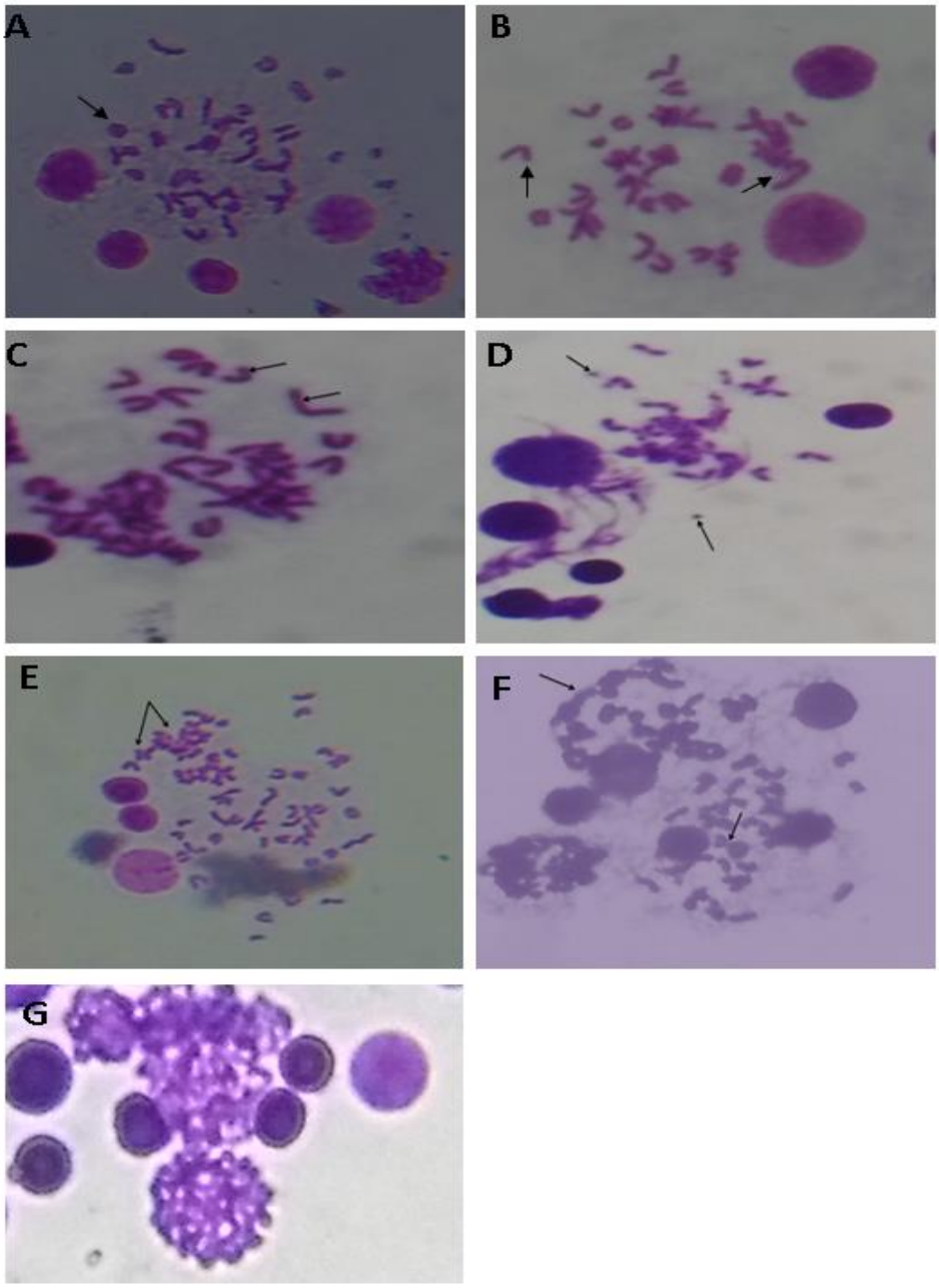

Fig. 1: Metaphase chromosome of mice bone marrow cells after iron sulfate treatment shows ring chromosome (A), chromatid deletions (B), chromatid deletions (C), fragment (D), stickiness (E), (F) Centric fusion and (G) polyploidy. Geimsa, x1000.

EVOO co-treatment significantly decreases the number of chromatid break. Meanwhile, the EVOO pre-treatment significantly decreases the number of fragment, chromatid deletion, and ring chromosome. There was no significant difference between the effect of EVOO either in cot-reatment or pretreatment.

The different types of chromosomal aberrations of the control and 48 hrs treated groups are presented in table 3. The most dominant type of chromosomal aberration at the 48 hrs treated groups (iron sulfate, iron sulfate + EVOO co-treatment and iron sulfate + EVOO pre-treatment) was fragment (20.8 ± 2.17, 16.0 ± 1.47 and 12.8 ± 1.80) respectively. On the contrary, chromatid break (5.75 ± 0.75, 2.25 ± 0.85 and 2.50 ± 1.04) was the lowest frequency for the 48 hrs treated groups (iron sulfate, iron sulfate + EVOO co-treatment and iron sulfate + EVOO pre-treatment) respectively. Pre-treatment with EVOO significantly lowered the number of the fragment which induced by iron sulfate.

T**able (2):** Aberrant cells, its percentage and different types of chromosomal aberrations in mice bone marrow cells treated with iron sulfate and/or EVOO after acute treatment for 24 hrs.

Groups*	Aberrant cells means ± SE	%	Types of chromosomal aberration				
			Fragment	Chromatid deletion	Chromatid break	Ring chromosomes	Stickiness
Control	8.50 ± 1.94^{c}	28.3	8.50 ± 0.65^{c}	8.50 ± 0.65^{c}	8.50 ± 0.65^{c}	8.50 ± 0.65^{c}	8.50 ± 0.65^{c}
EVOO	9.67 ± 1.45^{bc}	32.2	10.0 ± 2.65^{bc}	10.0 ± 2.65^{bc}	10.0 ± 2.65^{bc}	10.0 ± 2.65^{bc}	10.0 ± 2.65^{bc}
Iron sulfate	18.00 ± 1.15^{a}	60.0	23.0 ± 1.73^{a}	23.0 ± 1.73^{a}	23.0 ± 1.73^{a}	23.0 ± 1.73^{a}	23.0 ± 1.73^{a}
Iron sulfate + EVOO (co-treatment)	14.5 ± 1.55^{ab}	48.3	14.3 ± 2.02^{b}	14.3 ± 2.02^{b}	14.3 ± 2.02^{b}	14.3 ± 2.02^{b}	14.3 ± 2.02^{b}
Iron sulfate + EVOO (pre-treatment)	13.3 ± 1.93^{abc}	44.2	13.3 ± 1.70^{bc}	13.3 ± 1.70^{bc}	13.3 ± 1.70^{bc}	13.3 ± 1.70^{bc}	13.3 ± 1.70^{bc}

No. of examine cell / animal=30. * No. of animal / group= 4. Each value represent means ± standard errors. Means without a common superscript in the same column are significantly difference (P<0.05).

T**able (3):** Aberrant cells, its percentage and different types of chromosomal aberrations in mice bone marrow cells treated with iron sulfate and/or EVOO after acute treatment for 48 hrs.

Groups*	Aberrant cells means ± SE	%	Types of chromosomal aberration				
			Fragment	Chromatid deletion	Chromatid break	Ring chromosomes	Stickiness
Control	10.8 ± 1.49^{b}	35.8	20.0 ± 1.78^{ab}	11.0 ± 2.20^{a}	3.50 ± 1.19^{a}	10.0 ± 0.82^{a}	5.50 ± 1.19^{b}
EVOO	9.67 ± 1.86^{b}	32.2	10.0 ± 1.73^{c}	8.33 ± 2.33^{a}	2.33 ± 0.33^{a}	9.67 ± 4.18^{a}	0.00 ± 0.00^{b}
Iron sulfate	18.8 ± 1.11^{a}	62.5	20.8 ± 2.17^{a}	14.3 ± 1.89^{a}	5.75 ± 0.75^{a}	14.3 ± 1.11^{a}	11.5 ± 1.19^{a}
Iron sulfate + EVOO (co-treatment)	14.5 ± 1.55^{ab}	48.3	16.0 ± 1.47^{ab}	14.0 ± 1.68^{a}	2.25 ± 0.85^{a}	11.0 ± 1.58^{a}	14.5 ± 2.66^{a}
Iron sulfate + EVOO (pre-treatment)	12.0 ± 2.20^{b}	40.0	12.8 ± 1.80^{bc}	10.8 ± 2.17^{a}	2.50 ± 1.04^{a}	8.00 ± 1.96^{a}	6.75 ± 2.69^{ab}

No. of examine cell / animal=30. * No. of animal / group= 4. Each value represent means ± standard errors. Means without a common superscript in the same column are significantly difference (P<0.05).

3.1.2. Chronic treatment (six weeks)

Total aberrant metaphase cells and the different types of chromosomal aberrations of the six weeks treated groups are presented in table (4) and fig1A-G. The results showed that there were significant difference between the six weeks treated groups (iron sulfate and iron sulfate + EVOO co-treatment) and both control groups. However, there were no significant differences between the iron sulfate treated group and iron sulfate + EVOO co-treatment group.

Regarding the types of chromosomal aberrations of the six weeks treated groups, the most prominent type of chromosomal aberration in iron sulfate and iron sulfate + EVOO co-treatment were deletion (26.2 ± 1.40 and 26.0 ± 1.18) and fragment (26.2 ± 1.70 and 19.0 ± 1.98). On the other hand, polyploidy (1.50 ± 0.34 and 1.33 ± 0.42) and centric fusion (2.00 ± 0.52 and 2.17 ± 0.87) were the lowest frequencies in six weeks treated groups. EVOO showed some protective effect through significantly decreases the number of fragments, chromatid break, and ring chromosome.

3.2. Mitotic index

The results of mitotic activity in acute and chronic experiments are shown in tables (5). The data showed that there were no significant differences in mitotic activity in all groups after 24 hrs of treatment. However, there was a significant lowering in mitotic activity in bone marrow cells after 48 hrs in iron sulfate treated group compared with iron sulfate+EVOO co-treatment and pre-treatment groups. Mitotic activity in the chronic treatment of iron sulfate showed significant lowering when compared with the control groups.

Table (4): Aberrant cells, its percentage and different types of chromosomal aberrations in mice bone marrow cells treated with iron sulfate and/or EVOO after Chronic treatment for six weeks.

Groups*	Aberrant cells means ± SE	%	Types of chromosomal aberration						
			Fragment	Chromatid deletion	Chro-matid break	Ring chromosom-es	Stickiness	Centric fusion	Polyploi-dy
Control	11.8 ± 1.19^{b}	3 9.4	17.2±1.0 8^{b}	14.3 ±2.12^{b}	5.83 ± 0.95^{ab}	16.5 ± 2.28^{b}	8.83±1.2 5^{b}	0.67 ± 0.49^{a}	0.00 ± 0.00^{b}
EVOO	13.0 ± 0.89^{b}	4 3.3	14.0±1.3 7^{b}	15.5 ±1.41^{b}	5.33 ± 0.71^{ab}	15.0 ± 2.11^{b}	14.0±1.7 3^{ab}	0.33 ± 0.21^{a}	0.00 ± 0.00^{b}
Iron sulfate	19.5 ± 1.18^{a}	6 5.0	26.2±1.7 0^{a}	26.2 ±1.40^{a}	8.00 ± 1.06^{a}	24.2 ± 2.94^{a}	17.7 ±1.91^{a}	2.00 ± 0.52^{a}	1.50 ± 0.34^{a}
Iron sulfate + EVOO (co-treatme-nt)	17.3 ± 1.05^{a}	5 7.8	19.0±1.9 8^{b}	26.0 ±1.18^{a}	4.33 ± 0.88^{b}	15.7 ± 2.01^{b}	18.5 ±1.73^{a}	2.17 ± 0.87^{a}	1.33 ± 0.42^{a}

No. of examine cell / animal=30. * No. of animal / group= 6. Each value represent means ± standard errors
Means without a common superscript in the same column are significantly difference (P<0.05).

Table (5): Mitotic indices of treated mice with iron sulfate and/or EVOO after 24 and 48 hrs of acute treatment and six weeks of chronic treatment**.**

Groups	Acute treatment		Chronic treatment
	24 hours	48 hours	6 weeks
Control	1.92 ± 0.42[a]	1.38 ± 0.15[b]	5.26 ± 0.48[a]
EVOO	3.29 ± 0.89[a]	5.76 ± 2.06[a]	5.38 ± 0.82[a]
Iron sulfate	2.16 ± 0.59[a]	1.63 ± 0.25[b]	2.11 ± 0.29[b]
Iron sulfate + EVOO (co-treatment)	2.78 ± 0.75[a]	4.57 ± 1.48[a]	3.68 ± 0.70[ab]
Iron sulfate + EVOO (pre-treatment)	2.42 ± 0.76[a]	6.10 ± 1.12[a]	-

Total number of examined cells / animal = 1500 cells. No. of animal in each groups = 4 animals in acute treatment. No. of animal in each groups = 6 animals in chronic treatment. Means without a common superscript in the same column are significantly difference (P<0.05).

3.3. RAPD-PCR

Thirteen single random oligo deoxy ribonucleotide primers were used to generate PCR-amplified fragments as RAPD markers, from pooled genomic DNA of 14 groups. Only seven RAPD primers gave visible and reproducible bands, these primers are 115, 137, CH3, CH5, OPA4, OPA10 and OPA 12 (Table 6). The total number of produced bands, number of polymorphic bands and number of common bands in acute (24 hrs and 48 hrs) and chronic (chronic) treatments are shown in table (6) and a represented in figure (2A and B). Results presented in table (6) and figure (3 and 4) showed the genetic similarities index among the treated groups in 24 hrs, 48 hrs and 6 weeks treated groups. In acute treatment (24 hrs), the genetic similarity among groups as following: iron sulfate treatment and iron sulfate+ EVOO co-treated groups was 0.745, EVOO treatment and iron sulfate + EVOO pretreated groups was 0.688 and iron sulfate + EVOO co-treated and iron sulfate + EVOO pretreated was 0.647 (Table 6).

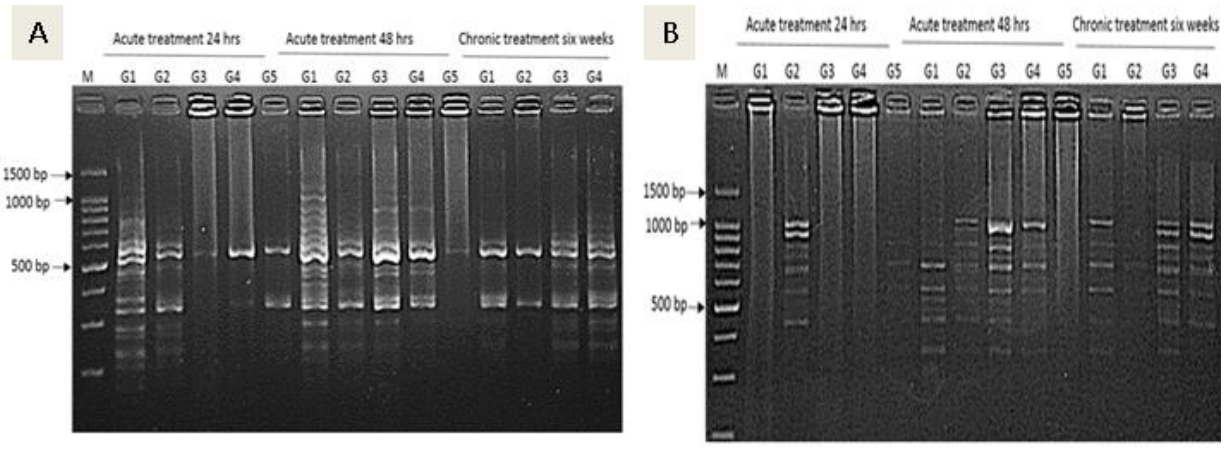

Fig. 2: Representative RAPD patterns produced with **(A)** primer OPA04 and **(B)** OPA10 primer using genomic DNA extracted from control, acute and chronic treated groups of mice. Molecular size marker (100bp ladder was loaded in lane M).

Table (6): Total number of bands, number of polymorphic bands and number of common bands after 24 and 48 hrs of acute treatment and six weeks of chronic treatment.

Prim-ers	PCR molecul-ear size	Acute treatment (24 hrs)			Acute treatment (48 hrs)			Chronic treatment (6 weeks)		
		Total No. of bands	Cm-mon bands	Polymor-phic bands	Total No. of bands	Com-mon bands	Polymor-phic bands	Total No. of bands	Comm-on bands	Polymo-rphic bands
115	400-1000 bp	7	1	6	5	4	1	6	4	2
137	200-1000 bp	19	4	15	21	12	9	12	10	2
CH3	400-1000 bp	9	1	8	6	3	3	9	2	7
CH5	300-1000 bp	5	0	5	5	1	4	3	2	1
OPA04	100-900 bp	20	4	16	22	5	17	20	6	14
OPA10	300-1000 bp	11	1	10	12	5	7	11	6	5
OPA12	400-1500 bp	7	1	6	4	0	4	6	1	5

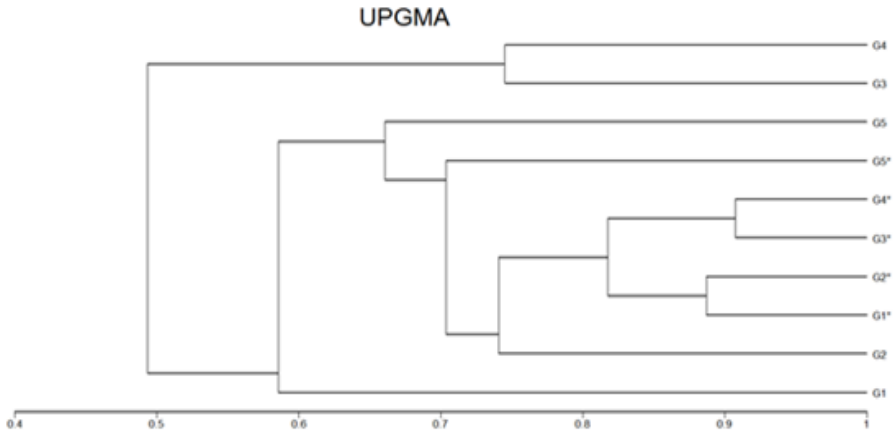

Fig. 3: Dendrogram using average linkage based on RAPD data analysis among the 24 hrs and 48 hrs treated groups. G1 = control group, G2= EVOO as positive control, G3= iron sulfate treated group and G4= Iron sulfate + EVOO co-treatment

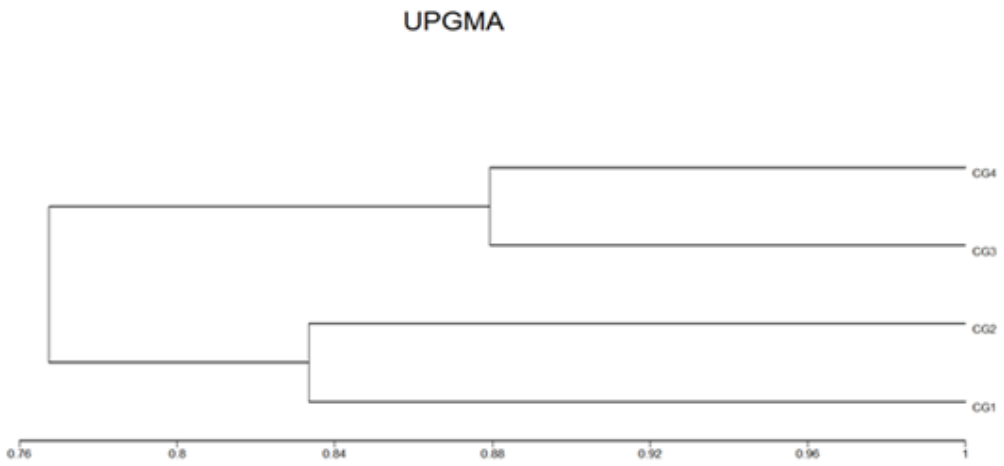

Fig. 4: Dendrogram using average linkage based on RAPD data analysis among the chronic treatment (six weeks) groups. G1 = control group, G2= EVOO as positive control, G3= iron sulfate treated group and G4= Iron sulfate + EVOO co-treatment.

4. DISCUSSION

The result of the present studies showed that the treatment of mice with iron sulfate induce chromosomal aberration. This aberration was represented by different types as fragments, chromatid deletion, chromatid break, centric fusion, ring chromosomes, stickiness, and polyploidy.

In addition, the data was shown that the frequencies of structural chromosomal aberrations were higher in iron sulfate than the frequencies of numerical chromosomal aberrations. Numerical chromosomal aberration (polyploidy) observed in bone marrow cells of chronic treatment only. However, structural aberrations observed in bone marrow cells of both acute and chronic treatments.

Treatment with EVOO either co-treatment or pre-treatment in acute treatment (24 hrs and 48 hrs) showing a decrease in aberrant cells percentage and decrease number of fragments. While in chronic treatment, EVOO has no effect on the number of aberrant cells, but it decreases the number of fragments, chromatid breaks and ring chromosome.

Many studies revealed that high levels of chromosome and chromatid aberrations were found in human lymphocytes and TK6 lymphoblast cells exposed to high-energy iron ions (56 Fe) (Evans et al., 2001; 2003 and Durante et al., 2002). Moreover, Iron induced free radicals and chromosomal aberration in rats' injected intraprotineal with $FeSO_4.7H_2O$ at dose (28.9 and 36.1 mg/kg $FeSO_4.7H_2O$) (Parveen and Shada, 2011). Also, there were significant increase of chromosomal aberrations and DNA damage induced by FeSO4 which given orally (200 mg Fe/kg) to rat (Parveen et al., 2014).

Chromosomal aberrations (chromosome deletion and chromosome break) were observed when exposed to 100 mg/ml of Co_3O_4 nanoparticles and Fe_2O_3 nanoparticles. The alteration in the antioxidant level caused chromosomal aberration and DNA damage in human lymphocytes (Rajiv, et al., 2016). On the other hands, low concentrations of either Fe_2 or Fe_3 (1.25, 2.5 and 5 mg/ ml) were not mutagenic in Chinese hamster ovary cells (CHO-9) treated in vitro, and the mitotic indices were also unaffected when compared to negative control cultures (Antunes et al., 2005), this may due to different iron dose and species.

Iron genotoxicity occurs may due to the presence of free iron in biological systems which is weakly bound is involved in generation of oxidative stress and ROS that leads to DNA damage and chromosomal abnormalities (Koskenkorva-Frank et al., 2013). The protection of EVOO against iron sulfate treatment showed a significant lowering of iron sulfate toxicity

when given one week before treatment with iron sulfate in acute treatment (24 hrs and 48 hrs) and low significant lowering in chronic treatment. O'Dowd, (2004) and Sarria et al., (2012 reported that olive oil phenolic compounds are the effective scavengers of superoxide, hydroxyl, and peroxyl radicals. Moreover, treatment with oral dose (2000 mg/kg) of hydroxytyrosol (phenolic substance naturally presence in olive oil) to rats not significantly enhance the number of aberrant cells or the mitotic index after 24 or 48 hrs compared to the positive control. Other studies showed that EVOO is effective at ameliorating DNA damage induced by Cr (VI) toxicity in rats (Khalil et al., 2013 and Saber et al., 2015). Furthermore, Ghorbel et al., (2015) reported that treatment with EVOO and its two fractions (OOLF, OOHF) revealed a protective effect against DNA damage induced by aluminum.

In this study pre-treatment with EVOO for one week before iron sulfate treatment, give significant protection against iron sulfate genotoxicity in acute treatment. This may due to EVOO contain a large number of antioxidants like phenolic compound and flavonoids (Montedoro et al., 1993; Brenes et al., 2000; Bianco et al., 2002 and P´erez-Trujillo et al., 2010). Another study, Horta et al., (2016) revealed that the pre-treatment with acerola juices 24 hrs before iron sulfate treatment showed anti-mutagenic activity, decreasing significantly micronucleus in bone marrow of mice and decrease DNA damage. Because acerola fruit has high vitamin C content, and large quantities of antioxidants, such as carotenoids, flavonoids, and anthocyanins (Chaves et al., 2004; Silva et al., 2014 and Nóbrega et al., 2015).

Co-administration of EVOO with acrylamide alleviated significantly hematological parameters, lipid and protein oxidation and the reduction of antioxidant status in erythrocytes. These beneficial effects of EVOO are mediated probably via its anti-oxidant power and the free radical scavenging activities of monounsaturated fatty acids, carotenoids, tocopherols, and polyphenols (Ghorbel, et al., 2017). Also, fullerene nanoparticles C_{60} and VOO (virgin olive oil) caused protection against cadmium chloride ($CdCl_2$) caused a reduction in the chromosomal number and several chromosomal aberrations in rats (Mulinacci, et al., 2018).

Genotoxic agents disrupt the integrity of the genome and also directly or indirectly affect the expression of DNA (Shugart and Theodorakis, 1994). These effects lead to an increase the incidence of different types of gene mutations and, result in genetic variability which detected by RAPD analysis. Changes in the band patterns reflect DNA alterations in the genome ranging from single base changes (point mutations) to complex chromosomal rearrangements (Atienzar et al., 1999 and 2002b). Thus, RAPD analysis offers a useful biomarker assay in ecotoxicology (Savva 1998 and Rocco et al. 2010 and 2012).

The genotoxic effect of iron sulfate on mice was investigated by using seven primers (115, 137, CH3, CH5, OPA04, OPA10, and OPA12), the RAPD analysis showed bands ranging in molecular size from 100 to 1500 bp with total band number is 85 bands. There was variation between its presence or absence and its intensity according to the treatment. Iron sulfate treatment produces the highest band changes and EVOO gives some protection against DNA alteration when given one week pre-treated with iron sulfate either in acute treatment 24 hrs or in acute treatment 48 hrs.

Other studies showed that DNA damage induced by heavy metals (Cd^{2+} and Cu^{2+}) was reflected by changes in RAPD profiles; disappearance of bands and appearance of new PCR products occurred in the profiles generated by kidney bean (phaseolus vulgaris) (Enan, 2006). Moreover, Qari, (2010) conducted a study to investigate the genotoxic and/or antigenotoxic effect of aqueous extract of the traditional plant Costus speciosus by using RAPD technique, the results demonstrated monomorphic numbers of genetic bands mostly, which were the electrophoretic products of PCR for all treatments compared with the control. Also, the results exhibited the ability of C. speciosus as antigenotoxic and anticytotoxic potential against EMS-induced DNA damage, cytotoxic and clastogenic effects in Allium cepa cells.

In addition, Rocco et al, (2014) revealed that changes in the RAPD patterns are expressed as decreases in genomic template stability (GTS), a measure reflecting the change in the number of RAPD profiles of Dicentrarchus labrax embryonic cell line (DLEC) generated by benzene and H_2O_2, in relation to profiles obtained from the non-treated cells. While, Salem et al., (2014) reported that, RAPD analysis was able to detect significant genetic alterations in roach Rutilus rutilus DNA, after contamination with a set of metals contained in the landfill leachates in comparison to a roach from a non-polluted reference pond, apparent changes were observed, such as appearances of some

new bands or the disappearance of bands as compared to the control.

The genotoxic effects of four heavy metal mixture (Fe, Cu, Cd, Pb) on Drosophila melanogaster was investigated by using six random primers, the results of RAPD analysis revealed bands ranging in molecular size from 100 to 3,000 bp, a total of 123 bands belonged to the control group, 153 new bands were formed, and 193 lost bands belonged to the treated group. The highest number of band changes was identified at the highest heavy metal mixture exposure on the 10th day of treatment in the fly tissues (Doğanlar et al., 2014).

Comparison between RAPD data as indicator of DNA fragmentation and the number of chromosomal fragment at both acute and chronic treatment, revealed similar result which indicate the protective effects of the pre-treatment of EVOO on iron sulfate genotoxicity indicated by significant reduction of chromosomal fragments at the pre-treated acute treatment (24 and 48 hrs). However, there was no significant protective effect of EVOO in chronic treatment with iron sulfate.

5. CONCLUSION

Iron sulfate in mice ration was able to produce structural chromosomal aberration in acute treatment and structural and numerical in chronic treatment. It also lowering in mitotic activity and induced DNA fragmentation (RAPD fragments) at both treatments. EVOO as a co-treatment or pre-treatment lead to decrease in chromosomal aberration especially chromatid breaks, chromatid deletion and fragments in acute treatment. In general EVOO as a co-treatment showed a significant role in lowering Iron sulfate genotoxicity in acute treatments.

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