African J. Biol. Sci., 16 (1): 21-32 (2020)
ISSN 1687-4870 e- ISSN 2314-5501 (online)
www.ajbs.journals.ekb.eg E.mail: aasdjournal@yahoo.com

# Radio-protective role of interferon and fenugreek on γ- radiation induced DNA instability

**Karima M.Sweify[1]; SaharA. Mosallam[1] ; Ibrahim A.Ibrahim[2] and Afaf H. Kamel[1]**
1- Women College for Arts, Science & Education, Ain Shams Univ.,Cairo Egypt
2- Atomic Energy Authority, Anshas.

**Received: January 15, 2020; Accepted: Feb. 20, 2020; Available online : March. 5, 2020**

## ABSTRACT

The radio-protective role of fenugreek (fen) and IFN was studied against the damage effects induced by gamma irradiation in liver of albino rats. Male and female albino rats were paired. The produced generations were separated into 3 classes PC, PT& P-ir, representing untreated, fed with standard food mixed with 5% Fen seed powder (FSP) and exposed to whole body irradiation (WBI) respectively. Animals were allowed for mating to give $F_1$. $F_1$ was separated into 3 subgroups (ir-ir), (ir+FSP) and (ir-IFN), subjected to another dose of irradiation, fed with standard food mixed with FSP, injected with IFN respectively. All individuals were arranged for pairing until the production of $F_2$. DNA assay was carried out using RAPD-PCR fingerprinting technique. Six arbitrary primers were used. They produced various numbers of fractions ranging between zero to 8 within each of the studied groups. Some specific fractions were picked out indicating polymorphic alleles. Quantitative mutations were observed within the percentage area of the generated bands. Highest and / or lowest similarity indices were observed between the studied groups indicating complementary to the used primer or pointing to some degree or to complete disturbance in the DNA sequencing as a result of the different treatments. In conclusion the remarked changes in DNA fingerprinting confirmed the potential transmission of radiation damage of genome to the progeny while each of fen and IFN ameliorated the harmful effects of irradiation.

**Keywords**: Radio-protective role, interferon, fenugreek, γ- radiation, induced DNA instability, albino rats.

## INTRODUCTION

Oxidative stress (OS) is a state of imbalance between generation of reactive oxygen species (ROS) and the level of antioxidant defense system. Radiation induced (OS) results in oxidation of protein, lipids and nucleotides (Sarhanand & Naoum, 2020). Therefore ionizing radiation is known to induce mutations and cell transformations through single and double strand DNA breakage leading to produce chromosomal instability and carcinogenesis (Vorobtsova, 2000; Bàlentovà *et al*., 2008; Abou-Zeid *et al*., 2018). Clear dose rate effects were observed in MNPCEs and CAs frequencies in mice and rats (Tanaka *et al* .,2008; Pillai & Devi, 2013;Bagheri *et al*.,2018). Also in human, an increase in MN frequency in hospital staff exposed to low dose of ionizing radiation was observed by Eken *et al*. (2010).

On the other hand, the expansive spread utilization of irradiation is drawing attention not only to its effects on exposed individuals, but also to the possible genetic damage transfer to the following generations. So, the trans-generation of genome instability from irradiated animals of $F_0$ to the $F_1$ and $F_2$ generations was extensively investigated bySlovinskà *et al*. (2004) and Bàlentovà *et al*. (2008). Their studies confirmed the potential

transmission of radiation damage to the progeny.

Recently protection against oxidative damage induced by radiation exposure is directed towards drugs of herbal origin due to their pharmacological properties and low toxicity (Hosseinimehr*et al*., 2007; Shaban *et al*., 2017). *Trigonella foenumgraecum* , commonly known as fenugreek (fen) and called Helba in Egypt, is a well known leguminous herb grown in India, Egypt and Middle Eastern countries. According to Lust (1986) fen is one of the oldest known medicinal plants in the recorded history. Its seeds are used as condiment in India, a supplement to wheat and maize for bread making. Fen seeds (fen S) have also used as herbal medicine in many parts of the world for their carminative, tonic and aphrodisiac effects (TavaKoli *et al*,2015).It was found that fen S are rich in protein, fat, carbohydrate , mucilaginous matter and saponins (Rao and Sharma 1987; Khater *et al*., 2016). Its leaves are consumed widely in India and other countries as a green leafy vegetable and are rich source of calcium, iron, ß-carotene and other vitamins (Sharma *et al*., 1996). Moreover its seeds contain tannic acid, fixed and volatile oils, diosgenin, alkaloids, trigonelline, trigocoumarin, trigomethy lcoumarin and steroid saponin. Fen S is widely used as a galactagogue (milk producing agent) by nursing mothers to increase inadequate breast milk supply (Fleiss,1988). Fen has been demonstrated to produce antinociceptive, anti-inflammatory and anti-pyretic effects (Parvizpar *et al*., 2006; Malviya *et al*., 2010). Further, aqueous extract and a gel fraction isolated from the seeds showed significant ulcer protective effects (Suja *et al*., 2002).

Furthermore, the antidiabetic properties of fen S have been reported in animal experiments (Xue*et al*., 2007; Shetty and Salimath, 2009; Khalil and Al-Daoude, 2019) and in human subjects (Sharma *et al*., 1990; Cicero *et al*., 2004). Also, many reports evaluated the antioxidant action of fens extracts in *vitro* and in *vivo* studies (Xue *et al*., 2011; Sindhu *et al*., 2012). In addition fen is reported to have hypocholesterolaemic effects (Belguith-Hadrich *et al*., 2013). Fen is now available in encapsulated forms and is prescribed as dietary supplements for control of hypercholesterolemia and diabetes by practitioners of alternative medicine (Cicero *et al*., 2004).

IFN have important effects on many aspects of physiology, inducing cell growth, cell motility and cell function (Tortorella *et al*., 2000). The radio-protective effects of IFN were studied in mice and in Chinese hamster ovary cells (CHO) (Cong *et al*., 1998; Bolzàn *et al*., 2002). IFN is well known as a mutagenic and anticancer agent (Carrillo *et al*., 2006; Yano, 2008). Guo *et al*. (2004) found that IFN –α induced antiviral replication cycle, leading to a reduction in viral protein synthesis and eventually inhibition of viral RNA amplification.

On the other hand, RAPD is proved to be a successful method for the detection of genomic instability. Many investigations were concerned with RAPD-PCR, as it was used as an alternative method for identification and differentiation of *Pasteurella-pheumotropica* – isolated from laboratory rodents- rather than the conventional bacteriologic methods (Kodjo *et al*., 1999). The interspecific diversity among 15 common fish species was evaluated (Alne-na-ei *et al*., 2004). The detection of genomic instability by RAPD in patients with laryngeal and pharyngeal squamous cell carcinoma was carried out (Hussein & Habib, 2004) . Moreover, genotoxicity of dioxins on the albino mice was estimated through RAPD-PCR (Hafiz & Hanafy, 2009).

The objective of the current work was to investigate the damaging effects of gamma –irradiation induced genomic instability in the parental rats and their progeny through RAPD-PCR. Further, to

investigate the protective role of fen and IFN.

## MATERIALS AND METHODS

**a- Animals:**

Mature male and female white rats from central animal house of the National Research Centre, Dokki, Giza, Egypt, were used. The animals received standard laboratory chow and tap water ad libitum. Room temperature and a cycle of 12h light/12 h dark was maintained. Rats were allowed to acclimate for at least one week.

**b- Treatments :**

Animals of the present experiments were treated with one of the following:

1- Whole body gamma irradiation (WBI) was carried out, at Middle Eastern Regional Radio-isotope Centre for Arab Countries, Dokki, Egypt. Animals were irradiated with a single WBI dose of 2 Gy by γ-rays from a $^{60}$ Co source, at a dose rate of 0.571 Gy/min.
2- Fen S were cleaned, dried and crushed into fine powder and mixed with the standard food, in a ratio of 5 % **(**Shetty & Salimath, 2009).
3- IFN (Egyferon , α IFN-2b) was purchased from local pharmacy. A dose of (6.5 x $10^5$ U/Kg b.wt) was injected i.p 3 times weekly for 6 weeks.

a- Pregnancy establishment. Females were placed in the cage of adult fertile males by a ratio of 4:1 overnight. Females exhibiting a vaginal plug of coagulated ejaculate were considered pregnant (Gasser *et al*., 1992). The day of birth was known by daily inspection of the cage.

**Experimental design:**

Animals were divided into 3 groups (G).

G1: considered as parent control (PC), were left without any treatment, only standard food and tap water.

G2: received standard food in addition to 5 % FSP, they served as PT.

G3: exposed to 2 Gy WBI and acted as P-ir.

Animals of each group were handled separately. The pregnant females in G1 were isolated until delivery, after weaning, neonatal rats ($F_1$) were exposed to WBI, acted as $F_1$C-ir. These irradiated animals were allowed for mating to obtain $F_2$ generations ($F_2$C-ir). Animals of G2 (PT) were followed until the production of $F_1$. The latter were divided into two subgroups:

a) subjected to WBI and were known as $F_1$T-ir.

b) were fed on standard food mixed with FSP 5 % and was known as ($F_1$TT).

Animals of both subgroups were followed until $F_2$ were produced, i.e $F_2$T-ir; $F_2$TT.

G3 rats (P-ir) were left until $F_1$ achievement ($F_1$-ir). The latter were divided into 3 subgroups: i) subjected to extra dose of γ –rays ($F_1$ir-ir).

ii) rats were fed with standard food mixed with FSP 5% $F_1$ir-T.iii) injected with IFN ($F_1$ir-IFN).

Animals of the 3 subgroups were left to grow, arranged for pairing until the achievement of $F_2$. So, $F_2$ir-ir, $F_2$ir-T, $F_2$ir-IFN animals were obtained.

**DNA extraction & RAPD-PCR**

Animals were autopsied, pieces of liver were taken from 8 groups, control male and female, PMC, PFC, $F_2$C-ir, $F_2$TT, $F_2$T-ir, $F_2$ir-ir, $F_2$ir-T and $F_2$ir-IFN and frozen until processing. The genomic DNA was extracted using GF-1DNA extraction kit following instructions of the user's guide.

**The RAPD-PCR reaction**

The PCR was performed for amplification of the genomic DNA according Hecimovic *et al.* (1997) and Rapley (1998). This reaction was carried out using the PCR kit- purchased from

Sigma-which contains all the reagents required represented in RED Taq Ready mix PCR reaction mix, with $MgCl_2$. The sequences of the used primers are: (5\-GAAACGGGTG-3\, 5\-CAATCGCCGT-3\, 5\GTGATCGCAG-3\, 5\TCTGTGCTGG-3\, 5\GACCGCTTGT-3\ and 5\-AGGGGTCTTG-3\.

The extracted DNA from livers of the present experimental rats was amplified using the mentioned primers. RAPD-PCR was carried out using the well known basic principle steps; denaturation at 95$^o$C, primer annealing at 36$^o$C, primer extension at 72$^o$C. At the end of the reaction, the temp was fixed at 72$^o$C for 10 min followed by keeping the amplicons at 4$^o$C.The amplified RAPD bands were separated by electrophoresis on 1% agarose gel containing ethidium bromide. After electrophoresis, the gel was visualized using UV-transilluminator and was photographed, then it was subjected to analysis via gel documentation system (Gel Pro- Analyzer, version 4.1).The similarity index (S.I) among the treated samples was calculated based on pairwise comparisons of primers using the formula ; $S.I=2N_{ab}/(N_a+N_b)$ ,Where $N_{ab}$ is the common bands to the individual "a" and "b" while $N_a$ and $N_b$ represent the total number of bands individual "a" and "b" respectively.The SI values were converted into G.d. using formula; G.d=1-S., (Nei & Li, 1979; Lynch and Milligan,1994).

## RESULTS AND DISCUSSION

Through the use of PCR it is now possible to amplify and analyze any DNA that can be isolated (Mertens & Hammersmith, 2007). In the current study six primers were used. Each primer produced different number of fractions ranging between 0:8 (Table 1). Primer 1 produced from 1:5 bands. Primer 2 gave from zero:4 bands. As for primer 3 from 2:7 fractions were generated, Primer 4 generates from zero:7 fractions. Primer 5 yields 2:7 fractions. Lastly, with primer 6 gave 2:8 fractions. Groups $F_2$TT and $F_2$ir-T gave no bands with primer no 2 and no.4 (Table 1 & Fig. 1).The interpretation of these results may be found in the review presented by NCB1 Service (2012). PCR is an enzymatic reaction, where the quality and concentration of template DNA, concentrations of PCR components, and the PCR cycling conditions may greatly influence the outcome. Thus the RAPD technique needs carefully developed laboratory protocols to be reproducible. So mismatches between the primer and the template may result in the total absence of PCR products as well as in a merely decreased amount of the product. Consequently, RAPD result can be difficult to interpret.

On the other hand, polymorphic DNA bands were picked out in some groups (Table 1). Thus insertion of 2 specific bands were spotted in each of PMC, PFC with primer no.1 and 5 , respectively (Fig. 2).The highest affected group was $F_2$ir-ir as one unique band was developed with primers no. 1 , 2 , 5 and 6 (Table 1). Further $F_2$C-ir gave 4 specific bands with primer 3; and one fraction for each of primers no. 2 & 6. $F_2$ir-IFN group gave one band with each of primer 3 and 4. The insertion of specific fractions pointed to mutagenic effects induced by γ-radiation in $F_0$ and transmitted to $F_2$ individuals through $F_1$ generation. These results run in a full agreement with that of Welsh *et al*. (1995); Hussein and Habib (2004); Khater *et al*.(2016). They cited that the presence or absence of RAPD bands is a reflection of natural genetic variation present in normal tissue DNA, while at responsive effects due to DNA damage or an euploidy it occurs as a result of genomic instability.

Additionally, quantitative mutations (Q.M) were observed between the percentage areas of some of the sharing bands belonging to various groups. The highest Q.M was spotted in between $F_2$T-ir and each of PFC, PMC, $F_2$ir-T and $F_2$ir-IFN in the samples reacted with primer 1,

Row No.15 (Table 2). Q.M. occurred between PFC & $F_2$C-ir and $F_2$ir-ir with primers 3, 5 and 6.The results revealed significant decrease in area percentage. Unexpectedly significant increase was spotted between PFC and each of $F_2$C-ir & $F_2$TT, in band no.1 with primer 5, as PFC =17.73 and $F_2$C-ir =37.32, and $F_2$TT =32.86. The percentage area may alter due to γ- radiation exposure of $F_0$ & $F_1$ and due to extra dose of Fen where the effect was transmitted to their progeny ($F_2$). Such observation is known as trans-generational effect (Bàlentovà *et al*., 2008).

Many studies pointed to the mutagenic transmission. Jagetia and Krishamurthy (1995) postulated that the mutagenic changes brought about by low dose radiation may be passed onto the next generations. Moreover many data provide supportive evidence that irradiated cells have a long term memory which is expressed in genetic instability some time later. This memory can also be manifested as hypersensitivity of the progeny of irradiated cells to mutagenic challenge (Vorobtsova, 2000). Later Luke *et al.* (1997) found that increasing doses (0.1:4 Gy) of γ-radiation, administered to males of the parental generation before mating resulted in an increased mutation frequency in bone marrow cells in the $F_1$ generation and the transfer of some genetic changes leading to inhibition of cell proliferation and to chromosomal aberrations and DNA fragmentation from irradiated males to their progeny. Dubrova *et al.* (2000) suggested that radiation-induced changes leading to genomic instability may be inherited by epigenetic alterations. Epigenetic mechanisms such as hypomethylation or de novo methylation are proposed to be the major contributor to the process of carcinogenesis (Jones & Baylin, 2002). Moreover, Kozurkova *et al*. (2007) demonstrated that some kinds of epigenetic changes can really be induced by radiation exposure.

Highest similarity index (S.I.) was recorded as a result of reaction between the used primers and the PCR products of the experimental studied groups. Most of the recorded cases of higher similarity indices were picked out between each of primers 1 2, 3 and 5 with groups exposed to γ –radiation and fed with fen or extra dose of fen as follows : $F_2$C-ir & $F_2$TT; $F_2$T-ir & PFC & PMC; $F_2$ir-T & PMC and $F_2$C-ir & $F_2$TT with primer nos.1, 2, 3 and 5, respectively. Highest S.I (1.0, 0.8, 0.67…….) was detected indicating complementary to the used primer. This result revealed the protective role of fen in modulating the harmful effects of irradiation that in turn led to complete complementary with the used primers.

Several studies reported that fen is a potent antioxidant agent. Thus Naidu *et al*. (2010) postulated that fen extract exhibited good free radicals scavenging activities. Also, Xue *et al*. (2011) cited that fen ameliorates oxidative stress in rat cells which may be due to its antioxidant potential.

Unexpectedly high S.I was concluded in samples of groups PFC, $F_2$ir-ir; $F_2$C-ir & $F_2$T.ir (Table 3); $F_2$T-ir and each of $F_2$ir-ir and $F_2$ir-IFN (Table 4) with that of primer 4 and 6, respectively. The unexpected high similarity indices between these mentioned groups are indicating that irradiation of $F_0$ and $F_1$ or administration of IFN could not affect the genetic distances between the provided samples, i.e. the harmful actions of γ –radiation which may be induced in $F_0$ and $F_1$ could not show themselves in $F_2$ progeny. These results could be attributed to miscarriage in factors that influence the specificity of the primers. These factors are (1) the length of the primer and (2) the annealing conditions for those primers. If a lower annealing temp ($55^0$C) is used, these primers may base-pair with similar but not perfectly complementary sequences. These cause additional PCR products and the presence of additional bands in the agarose

gel after electrophoresis. Some of these bands may be PCR products of things such as primer-dimer complexes that amplify themselves and hence are artifacts of the PCR process (Mertens & Hammersmith, 2007).

On the other hand, the highest similarity indices spotted in the mentioned groups agreed with the results of Sloviskà *et al*. (2004).They found that the cytogenetic effects of irradiation were less marked in the irradiated progeny of irradiated males as compared to irradiated progeny of non-irradiated male rats. They added, that finding can reflect an adaptive response of cells to radiation. In addition, Vance *et al*. (2002) suggested that the altered response to acute somatic irradiation which was observed in progeny with a history of radiation exposure is due to cellular reprogramming.

Lowest similarity indices (0.22, 0.29, 0.0) resulted between $F_2$ir-ir and $F_2$ir-IFN compared to PFC and /or PMC pointed to the increase in the G.d. The results indicated complete disturbances in the DNA sequencing which become uncomplimentary or could not match the sequences of the used primers. The disturbances in DNA constituents are attributed to the genotoxic effect of γ – radiation (Kang *et al*., 2006; Sadeeshkumar *et al*., 2019).

In conclusion the remarked instabilities in liver DNA of $F_2$ generations confirmed the potential transmission of radiation damage of genome from the parents to the progeny. The results pointed also to the protective role of fen and IFN as well, yet further studies on this subject are needed to validate this conclusion.

**Table (1): RAPD finger printing pattern as a result of gamma irradiation and administration of fenugreek and interferon.**

| Pr. No | | Groups | | | | | | | | Sh.b |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PFC | PMC | $F_2$C-ir | $F_2$TT | $F_2$T-ir | $F_2$ir-T | $F_2$ir-ir | $F_2$ir-IFN | |
| 1 | No.b<br>Sp. b<br>C .b | 2<br>-<br>1 | 4<br>2 | 1<br>- | 1<br>- | 2<br>- | 4<br>- | 5<br>1 | 5<br>- | 6 |
| 2 | No.b<br>Sp.b | 2<br>- | 2<br>- | 2<br>1 | zero<br>- | 4<br>- | zero<br>- | 3<br>1 | 3<br>- | 6 |
| 3 | No.b<br>Sp.b | 2<br>- | 3<br>- | 7<br>4 | 2<br>- | 3<br>- | 3<br>- | 2<br>- | 3<br>1 | 4 |
| 4 | No.b<br>Sp. b | 2 | 2<br>- | 7<br>- | zero<br>- | 5<br>- | zero<br>- | 4<br>- | 7<br>1 | 6 |
| 5 | No.b<br>Sp.b | 6<br>2 | 3<br>- | 2<br>- | 2<br>- | 4<br>- | 7<br>1 | 6<br>1 | 4<br>- | 8 |
| 6 | No.b<br>Sp.b | 2<br>- | 5<br>- | 8<br>- | 2<br>- | 6<br>- | 4<br>1 | 7<br>1 | 7<br>- | 9 |

No. b: number of bands sp. b: specific band e.b: common band
Pr. no: primer number Sh. B: sharing band

**Radio-protective role of interferon and fenugreek on γ- radiation induced DNA instability**

**Table (2): Quantitative mutation detected as a result of gamma irradiation and administration of fenugreek and IFN.**

| Pr. No | R. No | PFC | PMC | $F_2$C-ir | $F_2$TT | $F_2$T-ir | $F_2$ir-T | $F_2$ir-ir | $F_2$ir-IFN |
|---|---|---|---|---|---|---|---|---|---|
| 1 | R4 | - | - | - | - | 41.1 | 21.89 | - | 17.1 |
| | R15 | 61.77 | 32.24 | 100 | 100 | 58.9 | 38.29 | 26.88 | 27.4 |
| 2 | R4 | 53.81 | 50.75 | - | - | 21.1 | - | - | - |
| | R14 | - | - | 48.77 | - | - | - | 36.58 | - |
| 3 | R3 | - | 31.36 | 14.3 | 46.71 | 31.74 | 25.63 | 41.33 | 38.79 |
| | R4 | 49.43 | 35.72 | 15.12 | 53.29 | 36.61 | 29.24 | - | - |
| | R12 | 50.57 | 32.92 | 22.47 | - | - | 45.13 | 56.76 | - |
| 4 | No Q.m | | | | | | | | |
| 5 | R1 | 17.73 | 24.18 | 37.32 | 32.86 | 23.26 | 13.19 | 15.95 | - |
| | R8 | - | 42.58 | - | - | - | 13.84 | - | - |
| | R11 | - | - | - | - | - | 10.13 | 16.4 | 26.36 |
| | R12 | - | 13.42 | - | - | - | 15.8 | - | 30.62 |
| | R16 | - | - | 62.68 | 67.68 | 31.23 | 28.77 | 24.95 | - |
| 6 | R2 | - | 17.72 | 14.25 | 43.99 | - | - | - | - |
| | R4 | 58.15 | 32.06 | 12.38 | 56.01 | 19.49 | - | 16.01 | 18.34 |

Qm: quantitative R. No: Row number

**Table (3): Similarity indices and genetic distances calculated from samples reacted with primer no.4.**

**S.I**

| G.d | PFC | $F_2$C-ir | $F_2$TT | $F_2$T-ir | $F_2$ir-T | $F_2$ir-ir | $F_2$ir-IFN |
|---|---|---|---|---|---|---|---|
| PFC | - | 0.55 | 0.0 | 0.67 | 0.0 | 1.0 | 0.55 |
| $F_2$C-ir | 0.45 | - | 0.0 | 0.83 | 0.0 | 0.55 | 0.71 |
| $F_2$TT | 1.0 | 1.0 | - | 0.0 | 0.0 | 0.0 | 0.0 |
| $F_2$T-ir | 0.33 | 0.17 | 1.0 | - | 0.0 | 0.67 | 0.67 |
| $F_2$ir-T | 1.0 | 1.0 | 1.0 | 1.0 | - | 0.0 | 0.0 |
| $F_2$ir-ir | 0.0 | 0.45 | 1.0 | 0.33 | 1.0 | - | 0.55 |
| $F_2$ir-IFN | 0.45 | 0.29 | 1.0 | 0.33 | 1.0 | 0.45 | - |

**Table (IV): similarity indices and genetic distances calculated from samples reacted with primer no.6.**

**SI**

| G.d | PFC | $F_2$C-ir | $F_2$TT | $F_2$T-ir | $F_2$ir-T | $F_2$ir-ir | $F_2$ir-IFN |
|---|---|---|---|---|---|---|---|
| PFC | - | 0.2 | 0.5 | 0.5 | 0.33 | 0.44 | 0.44 |
| $F_2$C-ir | 0.8 | - | 0.4 | 0.57 | 0.33 | 0.4 | 0.67 |
| $F_2$TT | 0.5 | 0.6 | - | 0.25 | 0.0 | 0.22 | 0.22 |
| $F_2$T-ir | 0.5 | 0.43 | 0.75 | - | 0.6 | 0.77 | 0.77 |
| $F_2$ir-T | 0.67 | 0.67 | 1.0 | 0.4 | - | 0.73 | 0.73 |
| $F_2$ir-ir | 0.56 | 0.6 | 0.78 | 0.23 | 0.27 | - | 0.71 |
| $F_2$ir-IFN | 0.56 | 0.33 | 0.78 | 0.23 | 0.27 | 0.29 | - |

The shaded block represents the highest value for each S.I and G.d

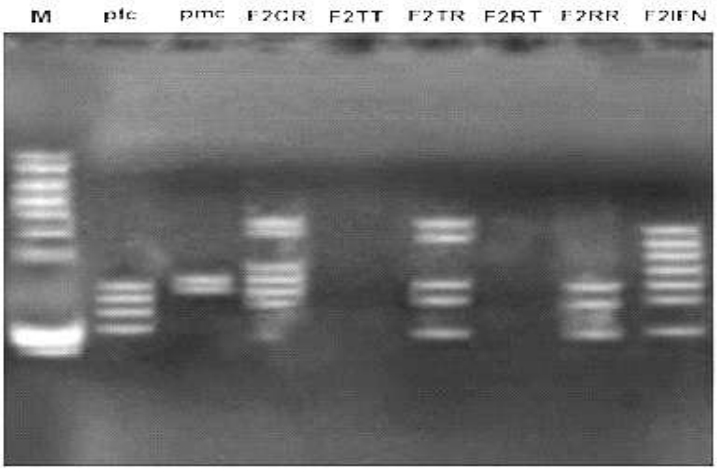


**Fig (1). RAPD-PCR fingerprinting generated by primer 5\TCTGTGCTGG3**

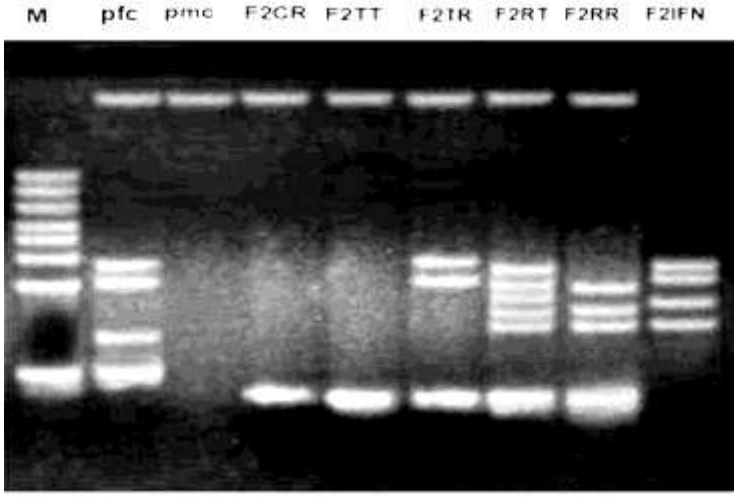


**Fig (2) RAPD-PCR fingerprinting generated by primer5\GACCGCTTGT3\.**

**M: Marker PMC: parent male control**
**PFC : Parent female control $F_2$C-ir: $F_2$ rats their grand parents were irradiated .**
**$F_2$T-ir :$F_2$ rats their grandparents receive food contain FSP ,their parent exposed to γ-radiation**
**$F_2$ir-ir: their grandparent and parent received γ-irradiation .**
**$F_2$ir-T: their grandparent exposed to γ-irradiation and their parent ate food contain FSP.**
**$F_2$TT: their grandparent and also their parent ate food + FSP.**
**$F_2$ir-IFN: their grandparent exposed to γ irradiation , their parent were injected IFN.**

## REFERENCES

Abou-Zeid, Sh. M.; EL-bialy, B.E.; EL-borai, N.B.; AbouBakr, H.O. and Elhadary, A. M.A. (2018). Radioprotective effect of date syrup on radiation-induced damage in rats. Sci. Rep., 8: 7423-7443.

Alne–na–ei, A.A.; Hassab, E.S. E.; Khallaf, E.A.;El-halfawy, K.A. and Abu-Mourad, I.M. (2004). Morphometry and DNA fingerprinting by RAPD –PCR of fish species in Bahr Shebeen Nilotic canal. Union Arab Biol. Cairo, Zool., 22(A):181-211.

Bagheri, H.;Rezapour, S.; Najafi, M.; Motevasli, E. and Mozdarani, H. (2018). Protection against

radiation-induced micronuclei in rat bone marrow erythrocytes by curcumin and selenium L-methionine. Iran. J. Med.Sci., 43 (6): 645-652.

Bàlentovà, S.; Slovinska, L.; Misurova, E.; Rybarova, S. and Adamkova, M. (2008). Effect of paternal rat irradiation transmitted to the progeny during prenatal development. Folia Biologica (Praha), 54:151-156.

Belguith-Hadriche, O.; Bouaziz, M.; Jamoussi, K.; Simmonds, M.; El-Feki, A. and Makni-Ayedi, F. (2013). Comparative study on hypocholesterolemic and antioxidant activities of various extracts of fenugreek seeds. J. F. Chem., 138:1448-1453.

Bolzàn , A.D.; Correa, M. and Bianchi, M.S. (2002). Effect of recombinant interferon– alpha on bleomycin-induced chromosome damage in hamster cells. Mutat. Res., 505:43-50.

Carrillo, M.C.; Álvarez, M.L. and Quiroga, A.D. (2006). Interferon alfa-2b triggers transforming growth factor –ß1 – induced apoptosis on preneoplastic liver ". Annals of Hematol., 5(4):244-250.

Cicero, A.F.; Derosa, G. and Gaddi, A. (2004). What do herbalists suggest to diabetic patients in order to improve glycemic control? Evaluation of scientific evidence and potential risks. Acta Diabetol., 41:91-98.

Cong , Xian-Ling ; Wang , Xian-Li .; Su, Qing ; Yan , S. and Cai , Lu (1998):" Protective effectsof extracted human-liver RNA , a known interferon inducer , against radiation induced cytogenetic damage in male mice. Toxicol. Lett., 94:189-198.

Dubrova, Y.E.; Plumb, M.; Gutierrez, B.; Boulton, E. and Jeffreys, A.J. (2000). Transgenerational mutation by radiation . Nature , 405(6782):37.

Eken, A.; Aydin, A.; Erdem, O.; Akay, C.; Sanal, H.T.; Saykut, B.; Sayal, A. and Somuncu, I. (2010). Cytogenetic analysis of peripheral blood lymphocytes of hospital staff occupationally exposed to low doses of ionizing radiation. Toxicol and Indus. H., 26(5):273-280.

Fleiss , P. (1988). Herbal remedies for the breast feeding mother.Mothering Summer, 68-71.

Gasser, D.I.; Yang, P. and Buetow, K.M. (1992). Palate teratogenicity and embryotoxicity of cyclo-sporine -A in mice. J. Cranofac .Genet. Dev. Biol., 12:55-58.

Guo, J.T.; Sohn, J.A.; Zhu, Q. and Seeger, C. (2004). Mechanism of the interferon alpha response against hepatitis C virus replicons. Virology, 35:71-88.

Hafiz , N.A. and Hanafy, Z.E.M. (2009). Genotoxic effects of dioxins on adult male albino mice. AL-Azhar Med. J., 38(2): 557-569.

Hecimovic, S.; Barisic, I.; Muller, A.; Petkovic, I.; Baric, I. and Ligutic, I. (1997). Expand long PCR for fragile X mutation detection. Clin. Genet, 52:147-154.

Hosseinimehr, S.J.; Azadbakht, M.; Mousavi, S.M.; Mahmoudzadeh, A. andAkhlaghpoor, S. (2007). Radioprotective effects of hawthorn fruit extract against gamma irradiation in mouse bone marrow cells. J. Radiat. Res. (Tokoyo), 48(1):63-68.

Hussein , A.A. and Habib, T.N. (2004). The detection of genomic instability by random amplified polymorphic DNAS (RAPDS) in patients with laryngeal and pharyngeal squamous cell carcinoma. J. Egypt. Ger. Soc.

Zool. Histol. & Histochem., (43c):197- 208.

Jagetia,G.C. and Krishnamurthy, H. (1995). Effect of low doses of gamma-radiation on the steady–state spermatogenesis of mouse: a flow–cytometric study. Mutat. Res., 332:97-107.

Jones, P.A. and Baylin, S.B. (2002). The fundamental role of epigenetic events in cancer. Nat. Rev. Genet., 3:415-428.

Kang , H. M.; Jang, J.J.; Langford, C.; Shin, S.H.; Park, S.Y. and Chung ,Y.J. (2006). DNA copy number alterations and expression of relevant genes in mouse thymic lymphomas induced by gamma-irradiation an N-Methyl-N-nitrosourea. Can. Genet and Cytogen ., 166(1):27-35.

Khalil, A. and Al-Daoude, A. (2019). Fractionated whole body gamma irradiation modulates the hepatic response in type II diabetes of high fat diet model rats. Mol. Biol Rep.,46(2): 2273-2283.

Khater,M. A.; El-Awadi, M. E.;Elashtokhy,M.A., Abdel-Baky,Y.R. and Shalaby.(2016):
"Physiological and molecular changes in Fenugreek (*Trigonellafoenum-graecum L.)*as a response to gamma rays."Int. J.PharmTech.Res.,9(12)306-316.

Kodjo, A.; Villard, L.; Veillet , F.; Escada, F.; Borges, E.; Maurin, F.; Bonnod, J. and Richard, Y. (1999). Identification by 16S r DNA fragment amplification & determination of genetic diversity by random amplified polymorphic DNA analysis of *Pasteurella pneumotropica* isolated from laboratory rodents. Lab. Anim. Sci., 49(1):49-53.

Kozurkova, M.; Letavayova, L. and Misurova, E. (2007). Influence of gamma irradiation on DNA methylation in liver of male rats and their offspring. Acta. Vet. Brno., 76:215-222.

Luke, G.A.; Riches, A.C. and Bryant, P.E. (1997). Genomic instability in haematopoietic cells of $F_1$ generation mice of irradiated male parents. Mutagenesis, 12:147-152.

Lust , J.B. (1986). The herb book, Bantam Books Inc., New York.

Lynch, M. and Milligan , B.G. (1994). Analysis of population genetic structure with RAPD markers. Mol. Ecol., 3:91-99.

Malviya, K.G.; Babhulkar, M.W.; Mail, P.Y. and Rangari, V.D. (2010). Evaluation of anti-inflammatory potential of *Trigonella foenum-graecum* (Fenugreek) seed extracts by using carrageenan induced rat paw edema. Drug Invention Today, 2(2):109-111.

Mertens, Th.R. and Hammersmith, R. (2007). Genetics Laboratory investigations. Pearson Prentice Hall p.193.

Naidu, M.M.; Shyamala, B.N.; Naik, J.P.; Sulochanama, G. and Srinivas, P.(2010).Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds . LWT. Food Sc. and Technol., 30:1-6.

Nei , M. and Li, W. S. (1979). Methaematical model for studying genetic variation in terms of restriction endonucleases. Proc, Natl. Acad.Sci.USA., 76:5269-5273.

Parvizpar, A.; Ahmadiani , A. and Kamalinejad, M. (2006).Probable role of spinal purinoceptors in the analgesic effect of *Trigonella foenum-greacum* (TFG) leaves extract. J. Ethnopharmacol.,104:108:112.

Pillai, T.G. and Devi, P.U. (2013). Mushroom beta glucan: potential candidate for post irradiation protection. Mutat. Res.,751:109-115.

Rao, P.U. and Sharma, R.D. (1987). An evaluation of protein quality of fenugreek seeds (*Trigonella foenum graecum*) and their supplementary effects. Food and Chem., 24:1-9.

Rapley, R. (1998). Polymerase chain reaction in molecular Biomethods, Hand-book (Rapley, R. and Walker, J.M., ed.), Humana , Totowa N.J,pp. 305-325.

Sadeeshkumar, V.; Duraikannu, A.; Aishwarya, T.; Jayaram, P.; Ravichandran, S. and Ganeshamurthy, R. (2019). Radioprotective efficacy of dieckol against gamma radiation-induced cellular damage in hepatocyte cells. Naunyn Schmiedebergs Arch. Pharmacol., 392(8): 1031-1041.

Sarhan, H.A.K. and Naoum, L.N. (2020). Protective role of Royal jelly against gamma radiation induced oxidative stress, cardio-toxicity and organ dysfunctions in male rats. Egypt. J. Hosp. Med., 78(1): 62- 67.

Shaban, N.Z.; Zahran, A.M.; El-Rashidy, F.H. and AbdoKodous, A.S. (2017). Protective effect role of hesperidin against γ-radiation-induced oxidative stress and apoptosis in rat testis. J, Biol. Res. (Thessalon), 24(5):5-15.

Sharma , R.D.; Raghuram, T.C. and Rao, N.S. (1990). Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. Eur. J.Clin. Nutr., 44:301-306.

Sharma , R. D. Sarkar , A. and Hazra , DK (1996):"Hypolipidaemic effect of fenugreek seeds :a chronic study in non –insulin dependent diabetic patient". Phytother Res .,10:332-334.

Shetty, A.K. and Salimath, P.V. (2009). Reno-protective effects of fenugreek (*Trigonella foenum graecum*) during experimental diabetes. e-SPEN, Eur. J. Cl. Nutr. & Metab., 4:137-142.

Sindhu, G.; Ratheesh , M.; Shyni , G.L.; Nambisan, B. and Helen, A. (2012). Anti-inflammatory and antioxidative effects of mucilage of *Trigonella Foenum greacum* (fenugreek) on adjuvant induced arthritic rats. Int. Immunopharmacol., 12:205-211.

Slovinskà, L.; Elbertová, A. and Mišùrovà, E. (2004). Transmission of genome damage from irradiated male rats to their progeny. Mutat. Res., 559:29-37.

Suja, P.R.; Anuradha, C.V. and Viswanathan, P. (2002). Gastroprotictive effect of fenugreek seeds (*Trigonella foenum graecum* ) on experimental gastric ulcer in rats. J. Ethanopharmacol., 81:393-397.

Tanaka, K.; Kohda, A.; Toyokawa, T.; Ichinohe, K. and Oghiso, Y. (2008).Chromosome aberration frequencies and chromosome instability in mice after long–term exposure to low-dose rat- γ irradiation. Mutat. Res.,657:19-25.

Tavakoli, M.B.; Kiani, A. and Roayaei, M. (2015). The effects of fenugreek on radiation induced toxicity for human blood T-cells in radiotherapy. J. Med. Signals Sens, (3): 176-181.

The NCBI Service Desk (2012). Random Amplified polymorphic DNA(RAPD) http:// www.nlm. Nih.gov./projects/ genome/probe/ doc/Tech RAPD.Shtml

Tortorella, D.; Gewurz , B.E.; Furman, M.H.; Schust , D.J. and Ploegh, H.L. (2000). Viral subversion of immune system. Annu. Rev. Immunol., 18:861-926.

Vance, M.M.; Baulch, J.E.; Raabe, O.G.; Wiley, L.M. and Overstreet, J.W. (2002). Cellular reprogramming in the $F_3$ mouse with parental $F_0$

radiation history. Int. J. Radiat. Biol., 78:513-526.

Vorobtsova , I.E. (2000). Irradiation of male rats increases the chromosomal sensitivity of progeny to genotoxic agents. Mutagenesis, 15(1):33-38.

Welsh, J.; Rampino, N.; Mcclelland, M. and Perucho, M.(1995). Nucleic acid fingerprinting by PCR –based methods applications to problems in aging and mutagenesis ". Mut. Res., 338:215- 229.

Xue , W.; Lei, J.; Li, X. and Zhang , R. (2011). *Trigonella Foenum greacum* seed extract protects kidney function and morphology in diabetic rats via its antioxidant activities. Nutr. Res., 31:555-562.

Xue, W.L.; Li, X.S.; Zhang, J.; Liu, Y.H.; Wang, Z.L. and Zhang, R.J. (2007). Effect of *Trigonella foenum graecum* (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotonic–induced diabetic rats. Asia . Pac. J. Clin. Nutr., 16:422-426.

Yano, H. (2008). Inhibitory function of interferon on hepatocarcinogenesis. Oncology, 75(1):22-29.

**الدور الواقى لكل من نبات الحلبة وعقارالأنترفيرون ضد عدم أتزان الدنا الناجم عن التعرض لأشعة جاما**

**كريمة محمد سويفي[1] –سحر عبد الرازق مسلم[1]ابراهيم ابو اليزيد ابراهيم[2]- عفاف هنداوي كامل[1]**

1-كلية البنات للآداب والعلوم والتربية جامعة عين شمس

3 - هيئة الطاقة الذرية –انشاص

**المستخلص**

يعتبر نبات الحلبة مشروب شعبي شائع الاستعمال له العديد من الفوائد المحسنة للحالة الصحية. أما عقار الإنترفيرون فيعتبر علاجا فعالا ضد الإصابات الفيروسية ، يحسن المناعة ويحد من تكاثر فيروس سى الكبدى. تهدف الدراسة الحالية الى محاولة معرفة الدور الوقائي لكل من مسحوق بذور الحلبة والإنترفيرون المصنع علي الآثار الناتجة من التعرض لأشعة جاما على المستوى البيولوچى الجزيئى لذكور واناث الجرذان ومواليدها من أفراد الجيل الثانى.

أجريت التجارب على عدد من الحرذان. حيث قسمت الحيوانات الى ثلاث مجموعات : PC -1 تستعمل كمجموعة ضابطة . PT -2 تغذى بالغذاء المتعارف علية مضاف اليه 5% من مسحوق بذورالحلبة. P-ir-3 تعرض للإشعاع (2Gy ). تركت المجموعات للتزاوج حتى انتاج افراد الجيل الأول $F_1$. تم تهريض أفراد الجيل الأول من المجموعة الضابطة للإشعاع لتنتج $F_1$C-ir والمجموعة الثانية جزء منها تعرض للإشعاع $F_1$T- ir والجزءالأخر اضيف الى غذائه مسحوق الحلبة $F_1$TT. المجموعة الثالثة P-ir قسمت الى ثلاثة اقسام :1- تعرض للإشعاع $F_1$ir-ir 2.- اضيف مسحوق الحلبة الى الغذاء $F_1$ir-T , 3- حقنت بالانترفيرون $F_1$ir-IFN. كل مجموعة تركت للتزاوج لتنتج أفراد الجيل الثانى.

اجريت التجارب على ألأفراد الناتجة من ابناء الجيل الثاني.حيث تم تقدير مدى عدم إتزان الدنا باستخدام دللالات التضخيم العشوائى لجزيئات الدنا متعدد الصورواستخدام تقنية سلسلة انزيم البلمرة RAPD-PCR. تم تحديد التغيرات التى حدثت فى دنا العينات قيد البحث باستخدام 6 بادئات.

تبين من النتائج أن كل بادئ قد أعطى عددا مختلفا من الأجزاء. تراوحت فى مجملها ما بين صفر الى 8 أجزاء . وقد كان من بينها أجزاء مشتركة فيما بين المجاميع المختلفة، وظهرت بعض الطفرات الكمية التي تمثلت في التفاوت بين النسبة المئوية للمساحة فيما بين تلك الأجزاء نتيجة للتعرض للتشعيع وكانت اقصاها في العينات التي تفاعلت مع البادئات ارقام1 ، 2 ( $f_2$-T-ir,PCF,PCM,$F_2$-ir-T,$F_2$-ir-IFN ) كما لوحظ بعض الأجزاء التى انحصر وجودها فى مجموعة دون الآخرى،مما يشير الي التغير في بعض الأليلات نتيجة لتأثير العوامل التي تعرضت لها الجرذان قيد البحث . من جهة آخرى وجد أن أعلى معامل للتشابه تراوح ما بين (1، 0.8 ، 0.67, الخ)، بين كل من البادئات ارقام 1، 2، 3، 5 مع المجاميع التي تعرضت للتشعيع وتغذت علي مسحوق الحلبة بجرعة واحده او جرعة اضافية علي حد سواء، مما يشير الي الدور الوقائي لبذور الحلبة ،كما يدل على أن التغيرات التى حدثت فى دنا تلك العينات تحدث عند نفس التتابع التى تتوافق مع تتابعات البادئ المستعمل . فى المقابل فإن أقل معامل للتشابه تراوح ما بين ( 0.22 ,0.29,الخ) ،حدث بين $F_2$ ir-ir,$F_2$-ir-IFN مقارنة بكل من PCF,PCM و ذلك يدل على أن حدوث اضطرابات واضحة فى تتابع الدنا مما جعله لا يتكامل مع تتابع البادئ المستعمل.

خلصت الدراسة الى أن التغيرات التى طرأت على الدنا المستخلص من اكباد جرذان الجيل الثانى تؤكد حدوث الطفرات فى جينوم الآباء و بالتالى انتقالها الى الاجيال التالية .كما تشير النتائج الى الدور الوقائى الذى احدثه مسحوق بذور الحلبة و عقار الانترفيرون.

# Radio-protective role of interferon and fenugreek on γ- radiation induced DNA instability