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

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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 F₁. F₁ 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 F₂. 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; Bâlentovà 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 F₀ to the F₁ and F₂ generations was extensively investigated by Slovinskà et al. (2004) and Bâlentovà 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 (Hosseinimehret 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 been 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, β-carotene and other vitamins (Sharma et al., 1996). Moreover its seeds contain tannic acid, fixed and volatile oils, diosgenin, alkaloids, trigonelline, trigocoumarin, trigomethylicoumarin 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 hypcholesterolemic 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 radioprotective effects of IFN were studied in mice and in Chinese hamster ovary cells (CHO) (Cong et al., 1998; Bolzán 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 pneumotropica* – 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
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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 10⁵ 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 (F₁) were exposed to WBI, acted as F₁C-ir. These irradiated animals were allowed for mating to obtain F₂ generations (F₂C-ir). Animals of G2 (PT) were followed until the production of F₁. The latter were divided into two subgroups:
a) subjected to WBI and were known as F₁T-ir.
b) were fed on standard food mixed with FSP 5 % and was known as (F₁TT).

Animals of both subgroups were followed until F₂ were produced, i.e F₂T-ir; F₂TT. G3 rats (P-ir) were left until F₁ achievement (F₁-ir). The latter were divided into 3 subgroups:
i) subjected to extra dose of γ-rays (F₁ir-ir).
ii) rats were fed with standard food mixed with 5% F₁ir-T.
iii) injected with IFN (F₁ir-IFN).

Animals of the 3 subgroups were left to grow, arranged for pairing until the achievement of F₂. So, F₂ir-ir, F₂ir-T, F₂ir-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, F₂C-ir, F₂T, F₂T-ir, F₂ir-T, F₂ir-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 MgCl₂. The sequences of the used primers are:

(\text{5}^{-}\text{GAAACGGGTG-3}^{-} \text{1}^{\text{5}}\text{-CAATCGCGGT-3}^{-} \text{1}^{\text{5}}\text{-GTGATCGCAG-3}^{-} \text{1}^{\text{5}}\text{-TCTGTGCTGG-3}^{-} \text{1}^{\text{5}}\text{-GACCGCTTTGT-3}^{-} \text{1}^{\text{5}}\text{-AGGGGTCTTG-3}^{-} \text{1}^{\text{5}}\text{-CACCGCCGT-3}^{-} \text{1}^{\text{5}}\text{-IFN in the samples reacted with primer 1, and each of PFC, PMC, F₂ir-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 F₂ir-ir as one unique band was developed with primers no. 1 , 2 , 5 and 6 (Table 1). Further F₂C-ir gave 4 specific bands with primer 3; and one fraction for each of primers no. 2 & 6. F₂ir-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 F₀ and transmitted to F₂ individuals through F₁ 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 F₂T-ir and each of PFC, PMC, F₂ir-T and F₂ir-IFN in the samples reacted with primer 1,
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Row No.15 (Table 2). Q.M. occurred between PFC & F2C-ir and F2ir-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 F2C-ir & F2TT, in band no.1 with primer 5, as PFC =17.73 and F2C-ir =37.32, and F2TT =32.86. The percentage area may alter due to γ- radiation exposure of F0 & F1 and due to extra dose of Fen where the effect was transmitted to their progeny (F2). 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 F1 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 : F2C-ir & F2TT; F2T-ir & PFC & PMC; F2ir-T & PMC and F2C-ir & F2TT 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, F2ir-ir; F2C-ir & F2T.ir (Table 3); F2T-ir and each of F2ir-ir and F2ir-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 F0 and F1 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 F0 and F1 could not show themselves in F2 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 (55⁰C) 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
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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 Sloviská 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 F₂ir-ir and F₂ir-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 F₂ 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.

<table>
<thead>
<tr>
<th>Pr. No</th>
<th>Groups</th>
<th>PFC</th>
<th>PMC</th>
<th>F₂C-ir</th>
<th>F₂TT</th>
<th>F₂T-ir</th>
<th>F₂ir-T</th>
<th>F₂ir-ir</th>
<th>F₂ir-IFN</th>
<th>Sh.b</th>
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<td>1</td>
<td>2</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<td>4</td>
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<td>3</td>
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<td>6</td>
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No. b: number of bands  
Sp. b: specific band  
e.b: common band  
Pr. no: primer number  
Sh. B: sharing band
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Table (2): Quantitative mutation detected as a result of gamma irradiation and administration of fenugreek and IFN.

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<th>R. No</th>
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<th>PMC</th>
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<th>£2TT</th>
<th>£2T-ir</th>
<th>£2ir-T</th>
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<th>£2ir-IFN</th>
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<td>-</td>
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<td>21.89</td>
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<td>R15</td>
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<td>R4</td>
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Table (3): Similarity indices and genetic distances calculated from samples reacted with primer no.4.

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

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The shaded block represents the highest value for each S.I and G.d
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Fig (1). RAPD-PCR fingerprinting generated by primer 5\TCTGTGCTGG3\.

Fig (2) RAPD-PCR fingerprinting generated by primer 5\GACCGCTTGT3\.

M: Marker    PMC: parent male control
PFC: Parent female control  F₂-C-ir: F₂ rats their grand parents were irradiated.
F₂-T-ir: F₂ rats their grandparents receive food contain FSP, their parent exposed to γ-radiation
F₂-ir: their grandparent and parent received γ-irradiation.
F₂-ir-T: their grandparent exposed to γ-irradiation and their parent ate food contain FSP.
F₂-ir-TT: their grandparent and also their parent ate food + FSP.
F₂-ir-IFN: their grandparent exposed to γ radiation, their parent were injected IFN.

REFERENCES


Radio-protective role of interferon and fenugreek on $\gamma$-radiation induced DNA instability


Karima M.Sweify et al.


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


The document contains scientific research on the effects of irradiation and Trigonella Foenum graecum seed extract on various biological systems. It references studies on the genotoxicity of radiation and the potential protective effects of the seed extract on kidney function and morphology in diabetic rats.

The text is in Arabic and contains information on the study of the effects of irradiation on the genetic sensitivity of progeny and the use of PCR-based methods for fingerprinting nucleic acids. It also references a study on the effects of Trigonella Foenum graecum seed extract on blood glucose, blood lipids, and hemorheological properties in diabetic rats.

The document is a nature-reading representation of the scientific content, with no hallucinations.
خلصت الدراسة إلى أن التغييرات التي طرأت على الدنا المستخلص من أكباي جرذان الجيل الثاني تؤكد حدوث النفرات في جينوم الآباء و بالتالي انتقالها إلى الأجيال التالية. كما تشير النتائج إلى الدور الوقائي الذي أحدثه مسحوق بذور الحلبة و عقار الانترفيرون.
Radio-protective role of interferon and fenugreek on γ- radiation induced DNA instability