Preclinical Study of Genuine Essiac Formula: A Cancer Treatment Eight-herbs' Tea Minimizes DNA Insult of X-rays

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Abstract

Context: Essiac tea is been used widely in the homeopathy market for cancer treatment. **Aims:** We hypothesized its use for DNA-damaged mitigation under very low ionizing radiation (IR) on BALB/c mice (10–40 mSv). **Settings and Design:** The radioprotection of Essiac tea formulae was evidenced by comet assay (CA) and micronucleus (MN) acridine orange staining. We also reported complete blood count, animal weight, and fasting glucose levels to control for tea toxicity. **Materials and Methods:** Fifty BALB/c male mice of 6-7 week old and pathogen free mice were randomly divided in to control group, control irradiated mice, irradiated and tea or ascorbic acid treated mice, tea treated mice and ascorbic acid treated mice. Genuine Essiac tea treatment was given *ad libitum* for 7 weeks and ascorbic for no >13 days. The animals were exposed to three different X-ray doses (10 mSv, 20 mSv, and 40 mSv). **Statistical Analysis Used:** An independent one-tailed *t*-test or Dunnett's test was used to compare animal weight, fasting glucose levels, white blood count, comet percentage, and MN percentage, between doses, treatment, and controls, after a Welch's ANOVA and Mann–Whitney U-test using Excel worksheets from Biostathandbook.com website. **Results:** The tea formula resulted in a significant reduction of DNA damaged evidenced by CA ($P < 0.01$ for dose 3–40 mSv). By MN staining, the peak of significant induction of MNs was by the lower doses, D1 and D2, with a P value = 0.001 and P value = 0.014, respectively; however those irradiated animals when were treated with tea showed reduction of MNs and no significant difference from controls. **Conclusions:** Using an optimized murine model, we demonstrated that Genuine Essiac tea is not toxic and that it acts as a radioprotector against very low doses of IR.

Keywords: *Cancer prevention, DNA damage, Essiac, reactive oxygen species scavenger, X-rays*

Introduction

Ionizing radiation (IR) sources today imply a well-recognized physical risk for living beings from all ranges of exposure. IR implementation in health services such as X-ray was presented as an originating factor of nonspecific lesions and various types of cancers to those within a proximal range. Exposure to the IR is common to certain people like professionals handling radioactive materials, to the patients undergoing radiodiagnostics and radiotherapy or as millions of people who travel by air and are exposed to security X-rays scanning every day. General population before the 1980s was exposed to natural environmental background sources (e.g., residential and radon, cosmic rays, and regional mining) which comprised the majority of IR exposures, but there is a dramatic increase in exposure by medical sources (such as in sterilization, tomography, nuclear medicine scans, dental imaging and radiotherapy), security systems, and new method for industrial production all of what have resulted in similar levels of population exposure from both environmental and medical radiation sources.[1-4] In several reports, it was described how not only medical technicians are expose to X-ray and occupational cancer risk but also patients and children.[2,5-9]

It is known that when IRs interact with living cells, almost instantaneously physical interactions take place, known as Rayleigh scattering, photoelectric, and the Compton effect, which are of relatively low energies. This stage constitutes the early effects or direct effects which produce reactive groups that can then break bonds between atoms. When IR interacts with living cells, there is also what it is called late effects and bystander effects.[10,11] All of these interactions are produced without

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preferences, no more than the distance of the interactions carried about molecules such as reactive oxygen species or reactive nitrogen species (ROS and RNS, respectively).[12] ROS are free radicals, reactive radicals, simply radical atoms, or groups of atoms that have one or more unpaired electrons, like O2− among others, and are able to react with other molecules in different ways. This happens from donating an unpaired electron to another molecule or snatches an electron in order to reach a stable state in cells.[13] ROS results in cellular aging, damages of its biomolecular components, cancer initiation, and other associated pathologies.[12,14-16]

In addition to the natural cell evolved clearance and DNA repair mechanisms, which are not the focus of the present study, there are substances which reduce the effects of IR in healthy tissues while maintaining sensitivity to radiation damage in tumor cells.[12,16] Several compounds have been reported to have radioprotection. These have been developed specially to reduce radiotoxicity, scavenge free radicals, and produce protection to nontumorous cells during radiotherapy in some types of cancers.[17-22] Due to increased awareness about radioactive substances and their fatal effects on human health, radioprotective agents are now the topic of vivid research for been agents also good as anticancer treatments as well as cancer prevention compound. There are numerous studies indicating plant-based therapeutics against cancer and radioprotection.[23-25] Such plants could be further explored for developing them as promising natural radioprotective agents with anticancer properties.[18,20,25-27] The majority of these radioprotective compounds fall into the category of antioxidants and have generated promising results by reducing xerostomia, mucus, pulmonary fibrosis, cystitis, inflammation, and alopecia, to mention a few observable effects with radiotherapy.[24,28,29] Because they already have mitigating effects that are scientifically proven, they can be taken as a reference to compare the anticipated results with respect to other new bioactive substances or radical scavengers.[26,30]

In the present study, we chose one known ROS-scavenging compound, ascorbic acid, or Vitamin C[21,31-33] to compare the radioprotection effect of a widely used tea in the homeopathy, dietary supplements, and cancer alternative treatment market. Genuine Essiac tea used in the present study has been used as a popular anticancer and antioxidant tonic, and we selected it due to the reported ROS-scavenging properties of some of the herbs which conform the Genuine Essiac formula.[30,34,35] We used the following Essiac tea composition to evaluate its effect at very low dose IRs on DNA damage: burdock root (*Arctium lappa*), sheep sorrel (*Rumex acetosella*), Turkish rhubarb (*Rheum palmatum*), slippery elm (*Ulmus rubra*), blessed thistle (*Cnicus benedictus*), kelp (*Laminariales*), watercress (*Nasturtium officinale*), and red clover (*Trifolium pratense*). We purchased from Home Bodies, LLC., the Genuine Essiac tea bag in a 1 lb (454 g) pulverized presentation.

Our interest is to study the effects of very low doses and dose rates of IR X type when it comes to living beings and establish a way to evaluate the radioprotection or radiomitigation of natural compounds. In this regard, we established an animal model to study stochastic IR effect produced by very low doses of X-ray. Although controversial results have been obtained for these very low doses of IR, they are reported to be a risk for leukemia and other cancer types.[5,6,36-39] We evidenced IR effects *in vivo,* and we illustrated the radioprotective actions of Genuine Essiac tea in whole body-irradiated BALB/c mice model.

The Genuine Essiac tea formula did not show any cytotoxicity at the used doses of administration, confirming previously reported results with similar formulas;[26,40] in addition, we not only demonstrated that Essiac tea is not toxic though it acts as a radioprotector of stochastic effect produced by very low doses of X-rays, doses to which could be exposed the personnel of image examination services, travelers, pilots, and security workers.[7-9,41-43]

Materials and Methods

Animals

Four-to-five-week-old pathogen-free BALB/c male mice (18–22 g) were provided by the Center for Comparative Medicine of the Faculty of Agronomy and Veterinary Medicine of the Universidad Nacional del Litoral (CMC-FAV-UNL). The animals were kept in pathogenfree facility and in wellventilated polypropylene cages under standard conditions of temperature (24°C ± 2°C), humidity (50% ± 5%), and given food (chow diet – Ganave SA) and water ad libitum. Different groups, on weeks 7 and 10 received different treatments, Essiac Genuine tea or ascorbic acid orally, respectively. The animals were housing with 12 h light/12 h dark cycles and rice hull bedding. Mice were handled in accordance with institutional guidelines. All animal experiments were approved by the Committee on Ethics and Safety of Experimental Work (CCT Santa Fe, CONICET), and handling was done according to the guidelines issued by the World Health Organization, Geneva, Switzerland, and the Argentina Legislation as well as the National Administration of Medicines, Food and Medical Technology, Argentina, Provision 6344/96.

Experimental mouse model procedures

The subsequent quarantine period lasted for 2 weeks. At 7 weeks of age, 56 mice were identified by cages and randomly divided into 11 groups. Group cages C5, 8, 10, and 11 did not receive any treatment, while on week 7, groups C1, 2, 4, and 7 (Genuine Essiac tea treatment) were given tea *ad libitum* for 7 weeks. From week 10, groups C3, 6, and 9 received *ad libitum* ascorbic acid water solution as 1 mg per animal per day for 13 days. All cage

groups with the exception of cages C7 and C11 on week 12 were exposed to three different X-ray doses: a dose D1 (10 mSv) for C4, 9, and 10; dose D2 (20 mSv) for C2, 6, and 8; and a dose D3 (40 mSv) for C1, 3, and 5.

Mouse weights were noted every 2 weeks, and the fasting blood glucose levels were register by Accu-Chek Guide (Roche) glucometer on tail-tip blood samples. Briefly, fasting glucose levels from a tail-tip blood drop were acquired using a needle G27 as a lancing device, and then, we touch the yellow edge of the test strip with the blood drop; the measures were given in mg/dl.

X-rays doses

On week 12, unanesthetized mice were restrained in a well-ventilated polypropylene box in batches of 5 animals and exposed to whole-body radiation from a Dynan AF500 X-ray machine (Dynan SA, Argentina) operating at 40 kV and 50 mA and a source-to-surface distance of 100 cm at the San Jose Hospital, Radiology Services, Diamante, Entre Rios, Argentina. To obtain the increasing doses, the shooting time were increased from 100, 200, and 400 msec. To measure the exposure rate of each radiation shot, a gas-filled analogical lecture detector was used. The corresponding effective dose (E) was of 10 mSv ± 3 mSv (D1), 20 mSv ± 4 mSv (D2), and 40 mSv ± 3 mSv (D3), respectively, in each mouse cage.

ROS-scavenging treatments

Essiac Tea

the Genuine Essiac tea bag in a 1 lb (454 g) pulverized presentation of burdock root (*A. lappa*), sheep sorrel (*R. acetosella*), Turkish rhubarb (*R. palmatum*), slippery elm (*U. rubra*), blessed thistle (*C. benedictus*), kelp (*Laminariales*), watercress (*N. officinale*), and red clover (*T. pratense*) was purchased from Home Bodies, LLC, and tea was cooked as suggested by the provider on glass Erlenmeyer container. Briefly, we bring to boil 1 L spring water, then we reduced the heat to medium and added the content of package, we go on simmer for 10 min, and finally, we incubated the tea at room temperature for 12 h. Although the provider did not suggest straining the herbs, we strain the herbs twice in order to give it to the mice *ad libitum* in a water container.

Ascorbic acid

ascorbic acid (Vacunace, 1 g ascorbic acid – Casasco SA, Argentina) was administered orally by dilution in water. Briefly, every day 5 mg of ascorbic acid was dissolved in 100 ml of drinking water and given to each cage, so the animals *ad libitum* received 1 mg per day per animal administration (30 µg/kg/day).

Sample collection and histological processing

Saphenous vein and tail-tip blood collection

On week 13, after 7 days from the irradiation, the distal one-half centimeter of the tail or the saphenous vein cleaned was pricked with a 21G needle, and a capillary pipette containing anticoagulants (ethylenediaminetetraacetic acid [EDTA]) was used to collect two samples of whole blood of approximately 25 µl each from the bleeding surface. The first sample was used for cell counting and micronucleus (MN) count, the second for comet assay (CA). Immediately after collection, the pricked surface was clean with a clot with 70% ethanol.

Manual white blood cell count

peripheral blood smear was prepared, air-dried, and fixed with absolute methanol for 15 min, the blood smear films then were stained with Wright-Giemsa and evaluated by a light Leica DM500 microscope, and the relative white blood count (WBC) count is reported by treatment at 7 days after irradiations and of 13-week-old mice.

Genotoxicity assays

Micronucleus count

MNs are extranuclear segments of chromatin that can arise as a result of two mechanisms: chromosomal breaks or DNA double breaks (clastogenesis) or disruption of the mitotic apparatus (aneugenesis). MNs are formed infrequently during mitosis of healthy cells along the erythropoiesis, which occurs in the bone marrow or spleen of adult rodents. After 6h erythroblasts excluded the nucleus of the final mitosis, if it is not the case, polychromatic erythrocytes (PCEs), basophilic cells that contain RNA in the cytoplasm are generating.In mice, micronucleated PCEs (MNPCEs) are not necessarily clean by spleen from peripheral blood. An elevated frequency of MNPCEs means a cytogenetic damage, chromosomal damage, and not necessary represents chromosomal losses, but also the result of DNA amplification, which is commonly observed in oncogenic process. Consequently, MNs % represent a more stable and latent genetic damage compared with the CA tailed DNA %.[44] After 7 days of irradiation and 13-week-old animals, peripheral blood samples were collected for WBC films. Peripheral blood films were distained by absolute methanol at 37°C for 1 h, followed by another distaining step of absolute ethanol at 37°C for 1 h. The slides were then stained with acridine orange and observed with a Leica DM500 fluorescence microscope using a ×100 objective. About 100 cells were scored per sample, and three slides from each experimental condition or treatment were study.

Comet assay-DNA single and double-strand breaks detection

CA is a classical technique to evaluate IR damage. Peripheral blood sample of 13weekold animals were collected as previously describe after 7 days of Xray radiation (dose 3 = 40 mSv), the samples were mixed 1:10 v/v with 1X PBS and were used for CA as the modified protocol from Simoniello *et al*.[45] Brieftly, 50 µl of cell suspension was mixed with 0.5% low melting

point agarose (LMPA, 100 µl), and load on 1% normal melting agarosecoated slides by duplicate, including all experimental conditions, and positive (H_2O_2 300 µM) controls. Finally, the third layer of 100 µl of LMPA was pipetted onto the cell-embedded agarose and allowed to gel at 4°C for 10 min. The prepared slides were immersed in freshly prepared cold lysis solution (0.40 ml of Triton X-100, 5 ml of dimethyl sulfoxide, and 40 ml of stock lysis solution with 2.5M NaCl, 100 mM EDTA, and 10 mM Tris, adjust to pH 10) and kept overnight at 4°C. After lysis, the slides were immersed in alkaline buffer (0.3N NaOH, 1 mM EDTA, pH 13) during 10 min for DNA unwinding and electrophoresed in the same buffer. Electrophoresis conditions were 10 min at 300 mA and 20 V (0.7 V/cm) and then neutralized in 0.4M Trizma base solution (pH = 7.5). Finally, samples were dehydrated in methanol and left to dry. Lyse, unwinding, and electrophoresis were conducted at 4°C, and the preparations were kept in the dark. All samples in addition to four positive samples (peroxide expose samples) were coded for "blind" analysis, dehydrated with stained solution of ethidium bromide (2 µg/ml), and comet images of 100 randomly selected cells from each of two replicated drops were scored from each sample under a Leica DM500 LED fluorescence microscope. Comet images were analyzed and registered using an Amscope digital camera 14 MP (Amscope, USA). Cells were classified as comet and not comet according to their tail presence (from no damage: Class 0 to damage: class 1), resulting in a single DNA damage score (damage or comet percentage) for each animal. Comet cell profiles with a small or nonexistent head and an extremely prominent and diffuse tail were not scored due to the possibility that they were apoptotic or necrotic cells.

Histology and tissue collections

On week 23, the animals were euthanized by cervical dislocation. All animals were submitted to necropsies. The liver and large and small intestines were collected and fixed in methanol for 2 h and then transferred to 70% ethanol and keep at −20°C. Collected tissues (liver and large and small intestines) were cut by half for paraffin embedded at room temperature and for future protein and DNA purification methods. We also collect blood from the heart: A 21G needle and a 3cc syringe were used to obtain 0.5 ml of blood from the left ventricle. The blood was then immediately transferred to 1.5 ml tubes and freeze for DNA purification.

Methanol-fixed paraffin-embedded tissue was stain by hematoxylin and eosin for morphology characterization.

Statistical analysis

For comet and MN analysis, data from 100 and 1000 cells per sample, respectively, were collected and assessed for significant ($P < 0.05$) increases in DNA damage and genotoxicity. An independent one-tailed *t*-test or Dunnett's test was used to compare each dose and treatment to the control, after a Welch's ANOVA and Mann–Whitney U-test. The same statistical tests were used to compare differences in animal weight, fasting glucose levels, WBC, comet percentage, and MN percentage, between all the experimental conditions (control, radiation-exposed, treated and radiation-exposed, and treated mice), using Excel worksheets from Biostathandbook.com website.

Results

Low doses and low rate doses to X-ray exposure

We evidenced DNA and chromosomal damage induced with different doses of X-ray from 10 to 40 mSv. The damage to cellular DNA *in vivo* induced by whole-body X-ray radiation exposure was accessed only in animals exposed to dose 3 (D3 = 40 mSv ± 5 mSv) by the modified alkaline CA. The animals showed at day 7 postradiation a significant increase of tail DNA percentage ($P = 0.003$), suggesting radiation-induced damage to DNA that can be still verified by CA after 7 days of the X-ray insult [Figure 1 and Table 1]. The percentage of tail DNA was significantly increased ($P < 0.001$) to 85% ± 5% by H_2O_2-positive control and 69% ± 5% by D3 exposure from comet percentage shown by control (24% ± 1%), tea treatment (8% ± 1%), and D3 plus tea treatment (13% ± 1%), while ascorbic acid did decrease D3 effect on mice to 46% ± 2%, which was not significantly different from IR D3 comet percentage. The administration of Genuine Essiac tea formula before radiation exposure resulted in a significant ($P < 0.01$) decrease in tail DNA percentage at the postradiation time period when compared with IR D3 alone group or ascorbic acid-treated and D3 irradiated mice.

There was evidence of cytogenetic damage by IR by MNs in peripheral blood samples. MNs were determined by acridine orange staining on fresh and methanol-fixed peripheral tail-tip blood smear at 7 days postradiation. The results are summarized in Figure 2 and Table 2. The peak of significant induction of MNs in young erythrocytes of peripheral blood was for the lowest dose, dose 1 (10 mSv ± 5 mSv) ($P = 0.001$) and dose 2 (20 mSv ± 5 mSv) ($P = 0.014$), while samples exposed with dose 3 (40 mSv ± 5 mSv) showed no significant differences in MN content from those in controls.

Regarding treatment with Genuine Essiac tea, we found no significant differences in comet or MN number compared

Table 1: Comet assay results: Tail DNA percentage (data) and standard errors

	Group names					
	CTL−	CTL+	D3	Essiac	D3 plus tea	D3 plus AA
Data	24	85	69	8	13	47
SE	0.73	0.83	0.72	0.56	0.64	1.67

SE: Standard error, CTL: Negative and positive controls

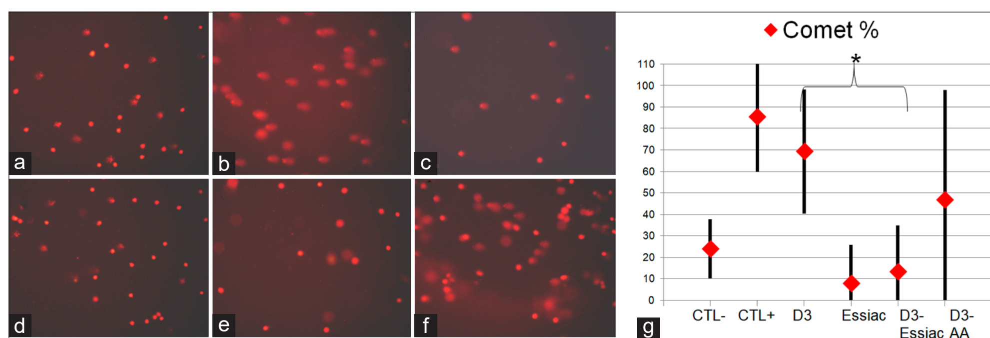

Figure 1: Comet assay: (a–f) Representative microphotographs of comet assay (Leica DM500 × 20). (a) negative control; (b) positive control (30 μM H_2O_2); (c) D3 = 40 mSv X-rays dose; (d) Genuine Essiac tea formula administer alone; (e) ionizing radiation-exposed samples under Genuine Essiac tea treatment; (f) exposed samples under ascorbic acid treatment (1 mg/per animal). (g) Scattered graphic with comet assay mean percentage of tailed DNA and standard deviation distribution. *Statistical significant differences from D3 irradiated mice

to controls. For ascorbic acid treatments, mice which were exposed to doses 2 and 3, showed no significant changes in MN (cytogenetic damage) % from that on the controls. However, ascorbic acid, in contrast to Essiac tea treatment in mice exposed to dose 1, showed significant differences and a higher number of MNs from the control indicating no improvement in DNA repair or DNA radioprotection by ascorbic acid at very low dose of IR. We have to underline that while ascorbic acid as an antioxidant directly reacts with reactive oxygen intermediates, dehydroascorbate, the oxidized form of ascorbic acid, has been reported to stimulate pentose phosphate pathways and glutathione levels that can suggest the induction of additional carbon and nitrogen sources for cell growth as can be glutamine. As well as other off-target features of AA, it has been reported that at high doses, it shows a pro-oxidant function.[46,47]

Ionizing radiation and ROS-scavenging compound effects on animal wealth

It is important to underline similar histological morphology between irradiated, treated, and control tissue samples for large intestine, small intestine, and liver tissue samples as well as no secondary effects of Genuine Essiac tea administration to the animal wealth determined by weight or fasting glucose levels. Ascorbic acid as well as Genuine Essiac tea was administered *ad libitum* in the freshwater supply. Ascorbic acid has been reported previously that at the supplemented dose showing no adverse effect in similar mouse strains, here, it was administered for no >14 days as previously suggested.[31] For this Genuine Essiac tea formula, on the other hand, there was no previous study, and it was administered daily *ad libitum* for 4 weeks. The tea resulted in lacking visible effects on the wealth of the animals as was tested by whole blood count, fasting glucose, and animal weight periodically. However, irradiation did have an effect on animal wealth. The total animal cohort did not show significant weight differences until 7 days postradiation, at this point, all of them showed a significant among group difference ($P = 0.042$) by a Welch's oneway analysis; and a Tukey– Kramer minimum significant difference assay showed that irradiated dose 3 samples were significantly lighter than animals receiving Genuine Essiac tea, irradiated or not. In addition, fasting glucose levels on the 2nd week postradiation were significantly different among groups ($P = 0.012$) though no group showed significant differences by Tukey–Kramer minimum significant difference or Dunnett's test.

Relative WBC performed 7 days after IR presented significant differences ($P < 0,02$) for K/μl, and a significant increase of WBC with respect to the control samples was for IR mice treated with Genuine Essiac tea and AA and all IR exposures (Dunnett's test $P < 0.048$). Although it was not a significant difference, Genuine Essiac tea administered alone showed a tendency for an increase of the WBC. In addition, neutrophil percentage showed a significant increase on all treatments from controls and tea alone (Welch's test $P < 0.01$), and by a Dunnett's test, only irradiated dose 1 animals treated with Essiac tea showed an important difference ($P = 0.05$). Regarding lymphocyte count, they showed significant changes (Welch's ANOVA $P = 0.011$). Ascorbic acid treatment showed an increase in lymphocyte count with levels similar to controls; all IR-exposed animals showed a nonsignificant decrease in lymphocyte percentage with respect to the controls. Genuine Essiac tea-treated animals showed a nonsignificant increase in lymphocyte portion with respect to controls and a significant increase with respect to all irradiated samples ($P < 0.03$). Finally, for basophils, monocytes, and eosinophils, portions showed no significant differences in their distribution, and only irradiated dose 3 samples showed a nonsignificant increase of monocytes and eosinophils.

Discussion

X rays and other ionizing radiation have been reported as a widely employed tool in diagnosis, radiotherapy, industry, and in custom security. However, due to its genotoxic nature to the proliferating cells and bystander effects, it is associated with some carcinogenic side effects through the direct or

Table 2: Micronucleus percentage (data) and standard deviation

	Group names										
	CTL	Essiac	D3	D2	D1	D3 plus tea	D2 plus tea	D1 plus tea	D3 plus AA	D2 plus AA	D1 plus AA
Data	4	6	6	13*	20*	4	7*	7*	10	9	18
SD	3	2	3	2	10	2	5	4	0	6	8

*Statistical significant differences from controls and treated mice from IR-exposed mice. IR: Ionizing radiation, CTL: Negative and positive controls

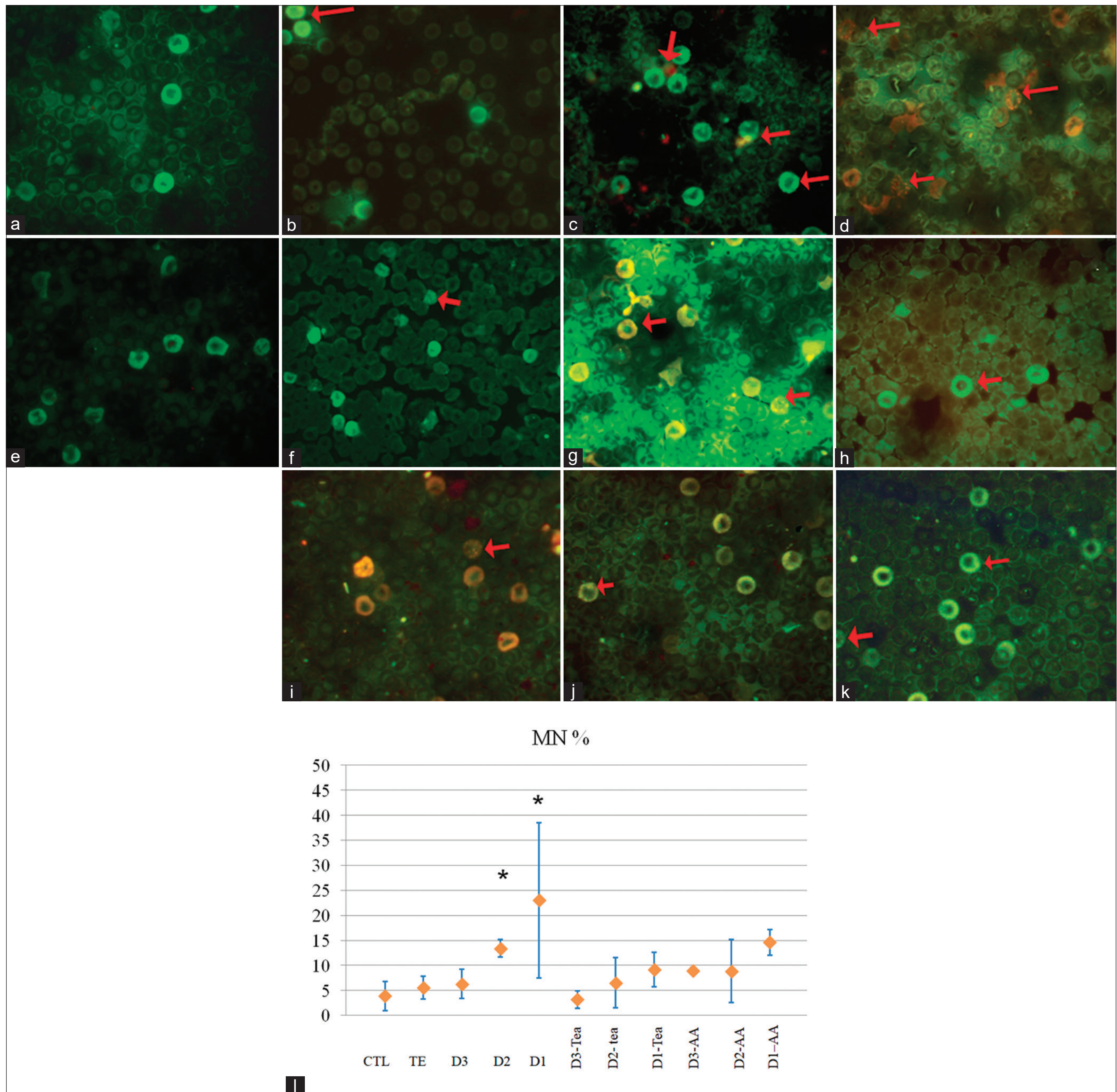

Figure 2: Micronucleus percentages. (a-k) Microphotographs at × 100 of acridine orange-stained peripheral blood smear showing micronucleated cells by arrows. (a) Control sample; (b) X-ray-irradiated sample dose 3 = 40 mSv; (c) X-ray-irradiated dose 2 = 20 mSv; (d) irradiated dose 1 = 10 mSv; (e) cells under tea treatment; (f) irradiated dose 3 under tea treatment; (g) irradiated dose 2 under tea treatment; (h) irradiated dose 1 under tea treatment; (i) irradiated dose 3 under ascorbic acid treatment; (j) irradiated dose 2 under ascorbic acid treatment; (k) irradiated dose 1 under ascorbic acid treatment. (l) Scattered graphic of micronucleus percentage mean and standard deviation. *Statistic significant differences from controls

indirect effects on DNA.[12] It is known that IR achieves their carcinogenic effect due to their involvement on the direct increase of ROS and RNS as well as DNA, protein, lipids, and different organic molecule breaks.[11,48]

X-rays are primarily a vital imaging tool used around the globe in health services as well as in security custom posts, and it is say that its risk are outweigh by its benefits. However, computed tomography scan corresponds to more than 1.5% of later in life cancer cases[41] where children showed more than a 20% or higher risk for leukemia and brain tumors later in life disease.[4,8] Other diagnostic X-ray exposures account for an early study for a 0.5% risk of cancer in adults.[9] However, cancer risk for lowdose exposure to other medical radiation tests and custom security post in airports are largely unknown.[42] Yet, if there are available new compounds to overcome its risk, staff members such as health caregivers, security officers, as well as pregnant women and especially pediatric patients and tourists would be all indeed undoubtedly benefit. Therefore, the quest for an effective, nontoxic compound with radioprotective capabilities is of immediate need, and this guided us into our interest in naturally occurring dietary antioxidants.

Few earlier studies on some of the medicinal teas and plants have indicated the usefulness of these natural products in reducing the radiation-induced genotoxicity and animal mortality.[13,21,26,27,32,40,41,48,49] The power to evidence the level of radioprotection or radiomitigation of medicinal teas is due to the variations in the type of DNA alterations that a particular test system detects; CA evidences basically the different types of DNA damage (double-strand breaks/single-strand breaks, etc.), while MN staining detects cytogenetic damage and fixed mutations which persist for at least one mitotic cycle and in mice for several days once released without selective removal by the spleen.[44]

X-ray insults and low-dose ionizing radiation in BALB/c mouse model (DNA damage detection)

The associations of MN assay and CA can be considered as a gold standard among mutagenic tests, and they have been used as established methods of the most reliable indicator of radiation-induced genetic damage.[11,50,51]

The present study was aimed to use a model by which we obtained repeatedly and accurately DNA damage by very low X-ray doses (<100 mSv) on BALB/c mice and we pursuit to understand the role of the natural Genuine Essiac formula tea in changing the level of radiation-induced DNA damage in blood peripheral hematopoietic cells.

In response to radiation ranges from 10 to 40 mSv, we evidenced DNA break damage and cytogenetic damage by CA and MN occurrence, the last we believe corresponded to chromosomal damage because these are the chromatin fragments/whole chromosomes that are not incorporated into the main nucleus during mitosis.[49] The MN assay, as an important endpoint for the assessment of cytogenetic damage, was also successfully used in previous studies with herbal compounds to assess their antigenotoxic potential.[52,53] Interestingly, lower dose (10 mSv) showed a higher number in MNs after 7 days of radiation exposure. While for DNA breaks, CA evidenced an increase in DNA tails percentage in dose 3 after 7 days of radiation exposure. As there are reports available stating that Genuine Essiac tea worked as ROS scavenger and IR (X-ray) exerts it damage by increasing cell ROS; we evaluated if its antioxidant feature protects cells against DNA damage induced by X-ray and exerts by ROS as previously reported for similar formulas.[26,30,35,54]

Eight-herb Essiac formula

Historically, Essiac tea is a combination of four to eight herbs that have proven to be a massive antioxidant and DNA protector. In a 2006 study, Essiac was put to test with different types of free radicals. Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$) and chromium oxidant Cr (VI) in RAW 264.7 cell lines and different hydroxyl radicals (OH−) were produced, xanthine/xanthine oxidase system was used to produce superoxide radicals (O2−), and the hydroxyl radicals (OH−) from previous reactions were used to cause cell membrane damage to initiate lipid peroxidation in order to measure malondialdehyde (MDA) production. In summary, the study indicated that Essiac is an OH− and O_2^- scavenger that acts as a protector from cell membrane damage by inhibiting lipid peroxidation caused by the ROS and other free radicals. Lipid peroxidation causes a cascade effect of lipidderived radicals, which produce additional damage to cell, like the aging process effect to cell membranes by MDA and other aldehyde groups, which are byproducts from lipid peroxidation, all these radicals may also produce DNA damage. All of these effects by free radicals are inhibited by Essiac and protect DNA from OH− radicals produced from Fenton reaction.[26,30] *A. lappa,* which conform the Essiac formula, have shown separately ROS-scavenging functions, apoptosis induction of tumor cells, and virus immune response enhancement.[26,55]

The present study showed that Essiac tea when administered orally *ad libitum* did not cause the significant formation of MNs or DNA tail comets indicating its nontoxic effect at the doses we used and administered for 28 days. However, there was a significant reduction in the MN frequency in the cells treated with Essiac tea compared with ascorbic acid (30 μg/kg/day) as well as a reduction on DNA tail percentage evidenced by CA. These observations clearly indicate the protective effect of Genuine Essiac tea formula as well as for AA against X-ray radiation-induced DNA damage in treated mice. Earlier studies from our laboratory have confirmed that with an acute exposure to 10–250 mSv whole body on week 8–12 of mice, a range of 40%–68% of DNA-tailed comet could be attained (data not shown). These findings are in good agreement with our and other earlier reports, where MN formation has been reported not only in the bone marrow erythrocytes post a carcinogenic insult but also on peripheral blood samples and especially with 100 mGy that have reported to not interfere with cell cycle.[22,56]

It is known that the number of MN yield depends on the frequency of the induction of chromosomal fragments, their

probability of exclusion at mitosis, proliferation status of cell population, cell cycle delay, and longevity of cells containing MNs.[10,14] Erythropoiesis is a continuous process, where the micronucleated erythrocytes are expected to die by apoptosis and macrophage clearance and to be cleared by spleen though this is not much the case in mice.[49,56,57] As MNs are more stable structure in mice, we suggest that the damage by higher doses is cleared early in the process of erythropoiesis as the damage is immediate by a more direct action on DNA bonds while with low X-ray doses the insult is produced by ROS mainly as a bystander effect of X-ray where the scholastic damage, in turn, would play a higher impact on bone marrow cells.[10] In addition, for high Xray doses the usual outcome is cell death, while middle or low doses of Xrays can insult cell at their different development stages, suffering DNA damage while proliferating or differentiating and the biological outcomes would be cell survival with mutations, or cell survival without mutations, depending on whether and how the cell repairs the damage and when it is detected.[14,15] As Ledebur and Schmid reported that the greater insult or the mutagenic effect on the bone marrow in mice the fewer the unmatured or damaged cells would enter the periphery blood as cell death would be generally the outcome, and for low doses of insult, the bystander X-ray effects could be evidenced later in periphery blood,[10,57] consequently the micronucleated erythrocytes would have better chances to be released to the periphery where spleen would not have an important clearance function in MN mice model as was reported previously.[44,49,58] The described scenario supports our findings of MN in periphery blood samples at the most low administered doses.

Could this reported feature be used for cancer prevention in people who are exposed to X-ray daily in their work environment as well as in patients undergoing radiotherapy?

Here, pretreatment with Essiac tea and AA reduced the frequency of radiation-induced MNs as well as DNA strand breaks in a mouse model. This inhibition of radiation-induced genetic damage by Essiac tea and AA is in agreement with other studies reporting that dietary ingredients such as Vitamin C and E protect against radiation-induced MN formation and DNA damage.[18,22,31,40,52]

Genuine Essiac tea formulae used here demonstrated to not be toxic to animals administered *ad libitum* and that it acts as a radioprotector against the DNA damage induced by very low IR (X-rays) doses on a mice model. The administered X-ray doses were similar to those staff or workers used to be exposed in diagnosis services and at custom security posts and are associated with what is called the stochastic effect of radiations.

Conclusions

The results from the present study suggest that Essiac tea more efficiently and effectively protects cells against radiation-induced genotoxicity. The mechanism of radioprotection by Essiac tea may be ascribed to its reported antioxidant, antilipid peroxidative, or free radical-scavenging properties.[26,30,35,40,55] Therefore, Genuine Essiac formulae demonstrated its potential as a radioprotective agents as it also showed no toxicity to the animal model. Furthermore, it needs more research in order to be prescribed for pregnant and pediatric patients though the commercial spot suggests administration to children. Our research opens the possibility of ESSIAC tea to be taken as a natural dairy supplement by Xray facilities operators and custom officers who are exposed to Xray from the custom scanning post.[19,20,42,54] Genuine Essiac formula, as dietary sources and antioxidant, would be easily tolerable; however, human studies need to be done to obtain their organically attainable concentrations to exert a real function in humans and more safely controlled dose in patients undergoing radiotherapy.

Finally, we optimized a murine model for further analysis of natural compounds and dietary supplements with promising radioprotection properties.

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Conflicts of interest

There are no conflicts of interest.

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