Original Article

The Protective Potency of *Medemia argun* (an Egyptian palm) Against Oxidative Stress and Tissue Injury Induced by Gamma Radiation in Rats

Mohamed Ebrahim Abdel-Alim,[1] Moussa H.R.[2], Fathy A. El-Saied[3], Moataz Ahmed Naguib Abd-Allah[3], Nesrein Saad Salim[1]

[1]Department of Biochemistry, Faculty of Agriculture, Benha University, Moshtohor, Egypt, [2]Radioisotope Department, Nuclear Research Center, Egyptian Atomic Energy Authority, Dokki, Giza 12311, Egypt, [3]Department of Chemistry, Faculty of Science, Menoufia University, Shebin El-Koom, Egypt

Abstract

Aim: Radiation affects all biological processes in the human body. *Medemia argun*(an Egyptian palm) is a mysterious plant from southern Egypt. In ancient Egypt, MA dried dates have been found in the famed tomb of Tutankhamun. Globally, this is the first study to look at the metabolomics and biological efficiency of Medemia argun(MA) in mitigating the risks of γ-irradiation. **Materials and Methods:** Rats were subjected to a single dose of γ-irradiation (6 Gy) for the whole body and injected intraperitoneally with or without MA ethanolic seed extract (200 mg/kg b. wt.). We studied the LD_{50} of MA ethanolic seed extract *in vivo* using male rats, liver function, antioxidant enzymes, inflammatory biomarkers, malondialdehyde (MDA), and nitric oxide (NO) to assess the biological functions and beneficial effects of the MA seed's ethanolic extract in reducing the γ-irradiation risks in rats. Also, DNA fragmentation was investigated via a Comet assay. Histopathological examinations were also performed. In addition, phytochemical analysis for MA was conducted. **Results:** The obtained results showed that 200 mg/kg b. wt. of MA ethanolic seed's extract is the recommended dose. γ-radiation increased DNA fragmentation, NO, MDA, inflammatory biomarkers, and liver function. Furthermore, there is a significant decrease in antioxidant enzymes, T. protein, and albumin. Conversely, MA (200 mg/kg b. wt.) treatment for 6 weeks effectively reflects most of the altered measurements induced by γ-radiation. Furthermore, histopathological examinations revealed that γ-radiation causes significant deleterious changes in the structure of liver tissue, whereas MA treatment preserves the cellular structure of the liver without the appearance of any changes. **Conclusions:** Our results showed that MA can be used as a healthy food during radiotherapy as a natural therapeutic drug due to its valuable nutritional benefits, safe nature, and low cost.

Keywords: Inflammatory biomarkers, *Medemia argun,* oxidative stress, radiotherapy, γ-irradiation

Introduction

Exposure to gamma radiation destroys living cells, according to two theories: Direct ionization of DNA and indirect ionization through overproduction of reactive nitrogen and reactive oxygen species, which end in cell death.[1,2] The danger of γ-irradiation stems from its interaction with biological macromolecules like proteins, lipids, and DNA via both indirect and direct pathways.[3,4] The indirect pathway is very important in increasing oxidative stress, leading to increased organ dysfunction due to cellular damage.[1,5] Serious troubles are typically encountered after acute exposure to excessive doses of gamma radiation.[6] Gamma radiation excites the radiolysis of water in the entire cellular system, which induces increased production of reactive oxygen species (ROS), which causes a serious redox imbalance and leads to an increase in oxidative stress in all cells and tissues.[7] Thereby, there is a great interest in protecting patients from the consequences of radiotherapy and specialized professional workers by developing natural antioxidant bio-drugs for the alleviation of radiation hazards. These bio-drugs are notably successful at scavenging ROS and, in consequence, improve

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Address for correspondence: Prof. Moussa H.R., Department of Radioisotope, Nuclear Research Center, Egyptian Atomic Energy Authority, Dokki, Giza 12311, Egypt.
E-mail: helal_moussa@hotmail.com

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the cellular/antioxidant balance, improving the immune defense system.[1] The study highlights the protective role of *Medemia argun* (MA) as a promising nutritional and functional ingredient in hampering most of the harmful radiotherapy-induced outcomes through its nutritional benefits, antioxidative properties, anti-inflammatory, anti-apoptotic, and cell regeneration abilities.[7-9] Such findings may offer an incentive for expanding its use during radiotherapy as a natural, safe, and low-cost therapeutic drug. Recently, scientist's interest in searching for natural compounds as antioxidants from plant substances has increased due to their ease of use, safe nature, and low cost to avoid the use of artificial compounds that have many poisonous side effects and very excessive costs.[2,8] In ancient times, the use of medicinal plants was a major source for treating human diseases. MA is one of the rare types of palm that belongs to the family *Arecaceae*, which grows in northern Sudan and southern Egypt (Nubian desert oases). It is the only type in the genus Medemia. The MA dried dates were found in the famed tomb of Tutankhamun in ancient Egypt.[10-12] Until now, the importance of the presence of dried palm fruits in the tombs of the ancient Egyptians was still ambiguous. The fruits of MA are sweet, and the flesh is thin and edible.[13] MA is very important desert plants, not only as natural sources of nutrients for human and animal nutrition but also as potential sources of antioxidant agents such as polyphenolics, including flavonoids and phenolics, in addition to other classes of secondary metabolites such as alkaloids, protoanthocyanidins (condensed tannins), and saponins.[7-9] A few studies have been conducted on the phytochemical analysis and biological activity of MA.[13,14] In the desert environment of Egypt, MA fruits are used and are an important source of good nutrition because they contain many important nutrients such as polyphenolic compounds (radical scavenging properties), B-complex vitamins, fibers, amino acids, essential minerals (K, Na, Ca, and Mg), carbohydrates, glucose, and fatty acids.[7] In the Nubian desert in Egypt, the fruits are used as a source of food for humans and mammals, where they are scarce.[9] Proanthocyanidins are a class of polyphenolic compounds identified from MA nuts and can be very useful as antioxidant, antitumor agents, and antimicrobial agents. They are also protective agents against the harmful impacts of oxidative stress, which causes serious diseases (neurodegenerative, cancer, and cardiovascular diseases), and against the oxidative and nutritional destruction of blood platelets and plasma components.[8,9,13,15,16] The proanthocyanidin fraction from Medemia nuts has distinct antioxidant properties and a high ability to protect biomolecules (proteins, lipids, and DNA) exposed to elevated levels of reactive oxygen and nitrogen species.[9,13] Globally, this is the first study related to the metabolomics, biological activity, and radioprotective role of MA as a functional food additive (a natural antioxidant) against oxidative stress and tissue injury induced by γ-radiation in rats. To evaluate the biological functions and beneficial effects of the MA seed's ethanolic extract in reducing the γ-irradiation risks to rats we studying the liver function (Alanine aminotransferase [ALT], Aspartate aminotransferase [AST], Gamma-glutamyl transferase [GGT], Alkaline phosphatase [ALP], Albumin, T. protein, and Alpha fetoprotein (AFP), antioxidant enzymes (Glutathione [GSH], Superoxide dismutase [SOD], and Catalase [CAT]), inflammatory biomarkers (Tumor necrosis factor-α [TNF-α], Heat shock protein [HSP70], Interleukin [IL]-6, IL-10, Caspase [CASP]-3,Monocyte chemoattractant protein (MCP-1), Matrix metalloproteinase-9 [MMP-9], 8-hydroxy-deoxyguanosine (8-OH-dg), MDA, and NO. Furthermore, DNA fragmentation was investigated through a genotoxicity test (Comet Assay). Histopathological examinations were also performed on the treated groups. In addition, phytochemical analysis for MA was conducted.

Materials and Methods

Plant material

In joint cooperation with researchers in the botanical garden, fresh and healthy fruits of MA were obtained from the Aswan University desert garden in October 2021, identified according to,[8,10] and dried in the dark at room temperature. The voucher specimens were saved in the Faculty of Science, Botany Department Herbarium, at Aswan University, Egypt.

Sample preparation and extraction of *Medemia argun* ethanolic seed extract

The extract was prepared by using the method of,[17] who reported that the seeds are separated from the fruits and placed in a clean and dry place for 5 days at room temperature until they are completely dry, then they are ground into fine particles by using an electric mill, then weighed, kept for 2 days in a conical glass flask containing 70% ethanol, and kept for 3 days in a shaker with continuous stirring at a rate of 150 rpm until mixing of active materials. Then filtering is done, and the extract is evaporated at 45°C. Eventually, the crude extract is weighed, collected in glass containers, and kept in a clean, dry place to be ready for use in any biological experiment.

Metabolomic profiling and antioxidants of *Medemia argun* ethanolic seed extract

Polyphenol fractions (flavonoids and phenolics) were evaluated by HPLC (Waters, USA) by the method of.[8] Proanthocyanidin was examined according to the methodology of.[15] Saponin was identified by the procedure in.[18] Crude protein content was measured using the method of.[7] Carbohydrate was measured by the method described in.[8]

Acute toxicity (LD_{50}) test for the *Medemia argun* ethanolic seed's extract

Acute toxicity was identified by the method in.[19] Briefly, 80 male, healthy Wister albino rats (180–200 g) were treated intraperitoneally with MA ethanolic seed extract at a concentration of 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 mg/kg b. wt. Saline solution (0.9% NaCl) is used as a resuscitation solution and/or conduit for MA extract daily for 1 week. Rats were carefully observed for any type of toxic symptom, and their mortality for 1 week was recorded if any occurred. The LD_{50} dose was calculated on the basis of maximum (survival) and minimum (death) levels for each dose as Equation 1:

$$LD_{50}: (M_0+M_1)/2 \quad \text{Equation (1)}$$

Whereas, M_0 (the maximum dose) of MA ethanolic seed's extract induced survival effects (no mortality), and M_1 (the minimum dose) induced death (mortality), as shown in Table 1.

Source of gamma-radiation

The source of γ-irradiation was a Gamma cell-40 irradiation unit (^{137}Cs), at the National Center for Radiation Science and Technology, Atomic Energy Authority, Egypt. The exposure rate was 0.84 Gy min^{-1}. Rats were exposed to 6 Gy of gamma radiation (a single dose for the whole body).[1]

Experimental design, animal treatment, and ethics potential

Sixty adult male (13-week-old) Wistar albino rats ($n = 15 \times 4$) weighing about 180-200 g were brought from the Research Institute of Ophthalmology (animal house), Egypt. Housed in cages (stainless steel) with good ventilated shields, the rats were acclimated to standard experiment conditions (26°C ± 2°C, 12/12 h light/dark, and 65% RH) for 1 week before the start of the experiment. During the whole experiment, all the animals were fed on a well-balanced diet (3.5% fats, 22% proteins, 0.55 table salt, 0.72% molasses, 0.25% vitamins, 60% corn maize, 5% wheat bran, 20% soybeans, and 10% growth additives) and had free access to drinking water *ad libitum* for 6 weeks.[1] Rats were randomly split into four groups. Group I: Represents control rats that received normal food and water daily for 6 weeks. Group II: Rats were subjected to gamma radiation (6 Gy), a single dose for the whole body after 1 week of the experiment. Group III: Rats were injected intraperitoneally with 200 mg/kg b. wt. of MA ethanolic seed's extract twice/week for 6 weeks. Group IV: Rats were subjected to gamma radiation (6 Gy), a single dose was administered for the whole body after 1 week of the experiment, then injected intraperitoneally with MA ethanolic seed extract (200 mg/kg b. wt.) twice a week for 6 weeks. Each group contains 15 rats. The animals in this experiment were subjected to the recommendations for proper care of experimental animals,[20] and specific local institutional protocols for the protection of animals under the supervision of authorized investigators were applied. The protocol was revised and approved by the Ethics Research Committee of the National Center for Radiation and Technology at the Egyptian Atomic Energy Authority in Cairo, Egypt (REC-NCRRT-34A/21).

Table 1: Estimation of the median lethal dose of the ethanolic crude extract of *Medemia argun* seed

Groups	Dose (mg/Kg b.wt)	Number of rats	Dead rats	Live rats	Dead (%)	Live (%)
1	50	8	0	8	0	100
2	100	8	0	8	0	100
3	150	8	0	8	0	100
4	200	8	0	8	0	100
5	250	8	0	8	0	100
6	300	8	0	8	0	100
7	350	8	2	6	25	75
8	400	8	4	4	50	50
9	450	8	6	2	75	25
10	500	8	6	2	75	25

Values are mean of samples in triplicate

Blood and tissue preparation

Six weeks after the experiment was completed, rats were lightly anesthetized with isoflurane for blood sampling according to the ethics statement,[21] and blood was collected from the internal jugular vein in special tubes. Blood samples were centrifuged at 4000–6000 rpm for 10–15 min; separated clear sera were kept at −20°C for further biochemical analysis. The rats were then sacrificed while under deep anesthesia. Immediately, carefully remove the liver from each rat. Tissue samples were divided into two parts for molecular, biochemical, and histopathological examination.

Genotoxicity test (comet assay)

The *in vivo* genotoxicity test was applied in different groups using single-cell gel electrophoresis analysis (Comet assay), which is a simple technique that is rapid and sensitive to reveal the degree of DNA fragmentation (damage).[22,23]

Biochemical analysis

Serum investigations

ALP, AST, GGT, and ALT activities, total protein, and albumin concentrations were calculated using a semi-automated analyzer (CCL-3001). Alpha-fetoprotein (α-fetoprotein) level was determined using a rat AFP ELISA Kit.[24] CASP-3 levels and 8-hydroxy-deoxyguanosine levels were assessed by an ELISA Kit obtained from Life Sciences Advanced Technologies in accordance with the manufacturer's requirements. The levels of TNF-α in rats were determined using the MyBioSource ELISA Kit (San Diego, CA, USA), Catalog no. MBS2507393. Matrix metalloproteinase-9 (MMP-9) was assessed using ELISA Kits (Rat MMP-9, Catalog no. E90553, China). Serum IL-10, monocyte chemoattractant protein-1 (MCP-1), and IL-6 were evaluated using the MyBioSource ELISA Kit (San Diego, CA, USA), Catalog no. MBS2700945, MBS8804486, and MBS4500393, respectively. Total HSP70 concentrations were measured by an ELISA Kit (Life Science Inc.). According to the manufacturer's recommendations, all measurements were performed.[1,24]

Liver tissue investigations

Nitric oxide (NO) was identified according to.[25] The level of malondialdehyde (MDA) was determined using a procedure.[26] Superoxide dismutase (SOD) was calculated by the method of.[27] Reduced glutathione (GSH) levels were measured according to.[28] Catalase (CAT) activity was determined by the method described in.[29]

Statistical analyses

Statistical analysis was performed with SPSS Version 17 statistic software package (SPSS Inc. Chicago, USA). Data were expressed as means ± standard error (SE). A significant difference (LSD) at $P \leq 0.05$.[30]

Results

Median lethal dose (LD_{50}) experiment

The estimated LD_{50} of MA ethanolic seed's extract was 400 mg/kg b. wt. Hence, half of this dose (200 mg/kg b. wt.) was considered the optimum dose (prophylactic dose) to be used during the experiment [Table 1]. The impact of the prophylactic dose (200 mg/kg b. wt.) on the different molecular, biochemical, and histological investigations was assessed [Table 1].

Phytochemical and metabolomic profiling was used to evaluate the biological activities of MA ethanolic seed extract. Metabolomics profiling reported that twenty-two polyphenols (phenolic and flavonoid fractions) were identified and their concentrations were evaluated using HPLC, as shown in Table 2. These polyphenols have an important role in human health.

Data as shown in Table 3 revealed the presence of many beneficial natural health compounds such as saponins, proteins, carbohydrates, and proanthocyanidins (condensed tannins) identified in the MA ethanolic seed extract

Determination of DNA damage by the Comet assay

The Comet assay is one of the most accurate techniques for the determination of adverse types of DNA damage. Using the Comet assay to reveal liver genotoxic potential induced by gamma irradiation showed significantly increased levels in the tail length of DNA ($P \leq 0.05$), tail moment, and tail intensity (DNA %) of irradiated rat's hepatocytes as compared to the control, indicating the adverse effect of γ-irradiation on DNA. On the other hand, rats that received an intraperitoneally administered injection of the ethanolic crude extract of MA (200 mg/kg b. wt.) significantly decreased the above parameters as compared to γ-irradiated rats, indicating the positive therapeutic impact of MA in repairing the fragmented DNA due to exposure to γ-irradiation [Table 4 and Figure 1].

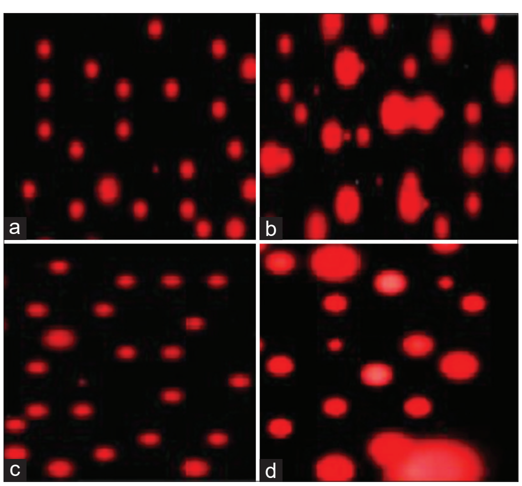

Figure 1: Photograph showing identification of DNA fragmentation in male rat liver using the Comet assay in the experimental groups: (a) control, (b) γ-irradiation (6 Gy), (c) *Medemia argun* extract, and (d) γ-irradiation (6 Gy) + *Medemia argun* extract

Histopathological assessment

For histopathological examination, the liver specimens were fixed in buffered formaldehyde (10%) for 24 h, slides were made by microtome (4 µM thickness) and then immersed in paraffin wax. Sections were stained with hematoxylin and eosin (H and E) and examined using a light microscope by the method of.[31]

As shown in Figure 2, the photograph identifies macroscopic manifestations in the livers of rats in different groups. (a) A

Table 2: Estimation of polyphenols (flavonoids and phenolics) in the *Medemia argun* ethanolic seed extract using HPLC

Compounds	RT (min)	Concentration (µg/g DW)
Phenolics		
Benzoic acid	1.1	83±4.23
Gallic acid	1.9	123±2.45
Resorcinol	2.1	115±4.52
Chloragenic acid	3.0	242±7.18
Caffeic acid	3.6	79±3.15
Vanillic acid	4.0	112±4.66
P-Coumaric acid	4.5	156±9.92
Salicylic acid	4.8	231±2.52
Ferulic acid	5.5	287±7.12
Sinapic acid	5.8	99±6.52
Cinnamic acid	6.1	116±8.91
Syringic acid	8.2	128±3.87
Rosmarinic acid	10.4	85±7.42
Flavonoids		
Catechin	1.1	98±2.39
Apigenin-7-glucoside	1.6	210±12.27
Kaempferol	2.3	142±11.84
Apigenin	2.4	267±17.32
Rutin	3.2	110±9.95
Chrysin	3.4	93±8.41
Hesperidin	4.9	77±6.56
Quercetin	6.9	91±5.52
Luteolin	9.0	68±2.29

Values are mean±SE of samples in triplicate. SE: Standard error, RT: Retention time

Table 3: Quantitative analysis of the phytochemical constituents *Medemia argun* seed

Parameters	Concentration (mg/100 g DW)
Saponins	1533±95.9
Protein	884±33.6
Carbohydrate	1718±64.3
Proanthocyanidin (condensed tannins)	956±39.4

Values are mean±SE of samples in triplicate. SE: Standard error regular, smooth-surfaced liver appears in a control rat. (b) Hepatotoxic rats have a large zone of ductular cholangiocellular proliferation (arrow) implanted inside fibrosis, as well as many irregular whitish micro-and macro-nodular structures on the liver surface.(c) A normal, smooth surface liver appears in a MA extract (hepatoprotective rat).(d) The liver of γ-irradiated + MA extract rats has a nearly normal smooth surface.

The effective role of MA in repairing the fragmented DNA and in alleviating the liver biochemical markers were confirmed by hepatic histopathological features. As a result, liver sections from γ-irradiation plus MA-treated rats showed a complete disappearance of the severe histopathological changes caused by γ-irradiation (6 Gy), with only a few inflammatory cells diffusing between the hepatocytes [Figure 3].

γ-irradiation (6 Gy) increased significantly ($P \leq 0.05$), in liver function (ALT, AST, GGT, ALP, and AFP), lipid peroxidation (MDA), NO, and inflammatory biomarkers (TNF-α, HSP70, IL-6, IL-10, 8-OH-dG, CASP-3, MCP-1, and MMP-9), in comparison with the control. While, γ-irradiation (6 Gy) induced a significantly ($P \leq 0.05$) lower level of GSH, CAT, SOD, T. protein, and albumin as compared to the control. Conversely, MA (200 mg/kg b. wt.) treatment for 6 weeks effectively reflects most of the altered measurements induced by γ-radiation [Table 5]. As illustrated in Table 5, γ-irradiation caused a significant increment ($P \leq 0.05$), in m-RNA transcript levels of HSP70 in liver tissues of rats in comparison with control rats and rats treated only with MA. However, γ-irradiation treated rats that were subsequently given MA (200 mg/kg b. wt.), showed a significantly decreased ($P \leq 0.05$) level of HSP70 expression in liver tissues as compared to γ-irradiation treated rats. γ-radiation induced a significantly increase $P \leq 0.05$ in 8-OH-dG and DNA fragmentation, as shown in Tables 4, 5 and Figure 1. The potent therapeutic efficacy of MA was manifested in repairing the DNA fragmentation induced by γ-irradiation and this improvement was confirmed by a decrease in the concentration of 8-OH-dG [Tables 4 and 5 and Figure 1]. As previously demonstrated, our data demonstrate the prophylactic potential of MA ethanolic seed extract on γ-irradiation to tolerate rats on multiple fronts

Table 4: Detection of genomic DNA damage in the different experimental rat groups

Parameters	Group I	Group II	Group III	Group IV
Tailed (%)	4±0.09^{c}	15±0.45^{a}	3±0.12^{c}	7±0.42^{b}
Untailed (%)	96±3.84^{a}	85±4.8^{c}	97±5.32^{a}	93±6.51^{b}
Tail length (μm)	1.41±0.03^{c}	6.72±0.27^{a}	1.36±0.05^{d}	3.05±0.15^{b}
Tail moment (unit)	2.17±0.06^{c}	47.91±1.62^{a}	2.04±0.06^{d}	9.66±0.67^{b}
Tail DNA (%)	1.54±0.04^{c}	7.13±0.36^{a}	1.50±0.04^{c}	3.17±0.13^{b}

Experimental groups: (GI) control, (GII) γ-irradiation (6 Gy), (GIII) *Medemia argun* extract, and (GIV) γ-irradiation (6 Gy) + *Medemia argun* extract. Values are mean±SE of samples in triplicate. Number with the same letter in each row are not significantly different at $P \leq 0.05$. SE: Standard error

Discussion

Radiolysis induced by gamma radiation generates a lot of ROS, which eventually severely damages living cells and causes organ dysfunction due to an imbalance between oxidants and antioxidants.[1,32] The phenomenon of oxidative stress is a consequence of the imbalance between the increased production and accumulation of ROS in tissues and the failure of the biological system to detoxify these reactive products, which

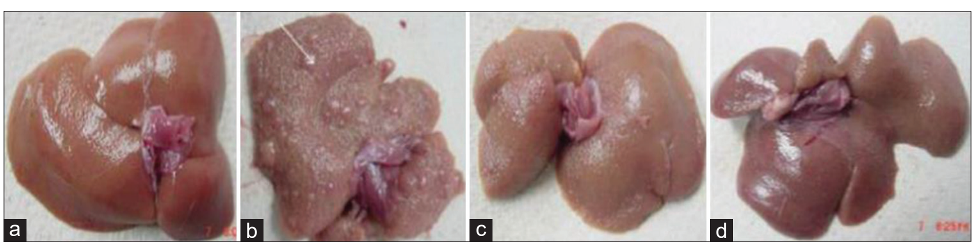

Figure 2: Photographs of morphological livers in different groups: (a) control, (b) γ-irradiation (6 Gy), (c) *Medemia argun* extract, and (d) γ-irradiation (6 Gy) + *Medemia argun* extract

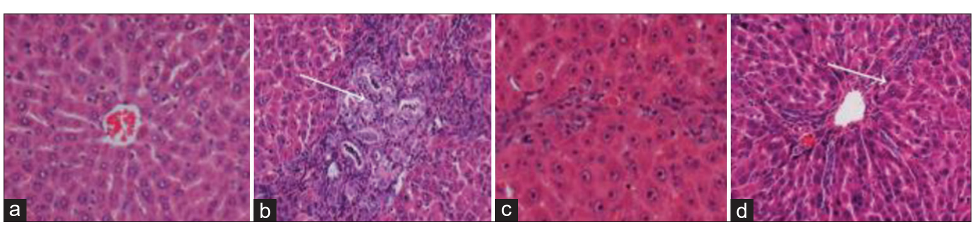

Figure 3: Histopathological sections of livers from different groups revealed: (a) control rats livers appeared to have normal architecture and histological structure; (b) γ-irradiated rats livers (hepatotoxic rats) showed the presence of pseudolobules with thick fibrotic septa and necrotic areas, and acute structural damage; (c) *Medemia argun* extract rats livers (hepatoprotective rat) showed moderate inflammation without fibrotic septa; (d) architecture with small zones of moderate necrosis and partially protected hepatocytes was observed in the livers of γ-irradiated + *Medemia argun* extract rats (Hematoxylin and Eosin stain original magnification: ×20)

Table 5: Changes in different biochemical parameters among the treated animal groups

Parameters	Group I	Group II	Group III	Group IV
TNF-α (pg/mL)	26±1.31[c]	75.3±3.02[a]	23±0.69[d]	37±1.85[b]
HSP70 (ng/mL)	7±0.21[c]	25±1.25[a]	5±0.16[c]	11±0.77[b]
IL-6 (pg/mL)	325±19.5[c]	573±32.2[a]	316±15.7[c]	390±23.7[b]
IL-10 (pg/mL)	408±16.3[c]	632±37.9[a]	398±27.1[c]	510±30.6[b]
CASP-3 (ng/100 mg tissue)	58±3.2[c]	171±10.3[a]	45±2.7[d]	73±6.1[b]
MCP-1 (pg/mL)	98±5.1[c]	302±18.3[a]	93±2.8[d]	145±7.5[b]
MMP-9 (ng/mL)	8±0.42[c]	31±1.81[a]	9±0.62[c]	13±0.79[b]
8-OH-dG (pg/mg tissue)	176±10.7[c]	289±14.4[a]	163±12.9[d]	197±11.7[b]
AFP (ng/mL)	5.13±0.16[c]	33.72±2.02[a]	4.73±0.29[c]	13.67±1.09[b]
ALT (U/L)	73±0.9[c]	164±2.5[a]	65±4.7[d]	92±4.6[b]
AST (U/L)	87±4.3[c]	195±11.7[a]	71±2.8[d]	108±9.6[b]
GGT (U/L)	36±1.8[c]	98±5.9[a]	29±1.7[d]	48±3.3[b]
ALP (U/L)	97±5.72[c]	236±18.5[a]	85±5.3[d]	120±7.1[b]
Albumin (g/dL)	4.73±0.44[a]	2.35±0.15[d]	4.53±0.19[b]	4.18±0.35[c]
Total protein (g/dL)	6.41±0.39[a]	3.98±0.17[c]	6.58±0.33[a]	5.27±0.28[b]
MDA (nM/g tissue)	12.9±0.78[c]	29.7±1.80[a]	11.8±0.84[c]	18.6±1.14[b]
NO (µM/mL)	0.97±0.12[c]	8.15±0.36[a]	0.69±0.04[d]	3.08±0.27[b]
SOD (U/g tissue)	98±4.9[b]	52±3.1[d]	102±9.1[a]	84±5.1[c]
GSH (U/g tissue)	128±8.9[a]	84±7.2[d]	112±6.6[b]	99±5.6[c]
CAT (U/g tissue)	85±5.3[a]	52±3.1[c]	88±6.1[a]	71±4.1[b]

Experimental groups: (GI) control, (GII) γ-irradiation (6 Gy), (GIII) *Medemia argun* extract, and (GIV) γ-irradiation (6 Gy) + *Medemia argun* extract. Values are mean±SE of samples in triplicate. Number with the same letter in each row are not significantly different at $P \leq 0.05$. CAT: Catalase, TNF-α: Tumor necrosis factor-α, HSP: Heat shock protein, IL: Interleukin, CASP: Caspase, MMP: Matrix metalloproteinase, 8-OH-dg: 8-hydroxy-deoxyguanosine, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase, ALP: Alkaline phosphatase, MDA: Malondialdehyde, SOD: Superoxide dismutase, GSH: Glutathione, SE: Standard error, NO: Nitric oxide, ALP: Alpha fetoprotein, MCP: Monocyte chemoattractant protein

leads to high damage and destruction of living tissues.[9] In recent years, many antioxidants have been used for their supposed or actually beneficial effect against oxidative stress, such as polyphenols, saponins, and Vitamin E.[33] In the Nubian Desert in southern Egypt and northern Sudan, a rare type of fan palm *(*MA) is found,[10,11,34] which has ecological importance as it provides shelter and food for desert wildlife. In ancient Egypt, the fruits of these palms were of great nutritional value, as evidenced by their presence in offerings in tombs.[11,12] It appears that the many significant medicinal benefits of MA extract to reduce the adverse effects that occur in rats as a result of gamma-radiation exposure may be attributable to the solidarity activity of the strong antioxidant polyphenols (phenolic and flavonoid) as well as other additional bioactive secondary metabolites, which are clearly capable of scavenging free radicals and thus highly improving cellular redox/antioxidant balance, increasing immune defense systems, and ultimately downregulating the signaling pathways that trigger the initiation of pro-inflammatory mediator cytokines and health-damaging programmed proteins.[7-9] The median lethal dose (LD_{50}) for MA ethanolic seed's extract of 200 mg/kg b. wt. was similar to that obtained by.[35] In this study, polar solvents, such as ethanol, were used because they are more efficient than other nonpolar solvents and water in extracting antioxidant compounds from MA. Twenty-two fractions of polyphenols (phenolic and flavonoid). MA contains a range of other bioactive secondary metabolites with important, beneficial, and antioxidant properties such as saponins, proteins, proanthocyanidins (condensed tannins), and carbohydrates, which are similar to those of.[7-9] MA is rich with proanthocyanidins that contain subunits (catechin, afzelechin, and gallocatechin). The high therapeutic value of MA may be related to the occurrence of high concentrations of many polyphenol fractions, which are an integral part of the human diet and have important and beneficial effects on human health and disease resistance.[9,13,15] Proanthocyanidins have important and promising antioxidant characteristics and may protect and preserve biomolecules (proteins, DNA, and lipids) exposed to free nitrogen ions, including peroxynitrite ($ONOO^-$) and free oxygen radicals. Also, as a significant and important protective factor against oxidative/nitrifying stress associated with many harmful and different diseases, such as neurodegeneration, cardiovascular disease, and cancer.[15] Antioxidant polyphenols (radical scavenging properties) in MA, which are characterized as being natural and having many health benefits, can be recommended for use in the food industry (as important and useful nutritional supplements) instead of artificial antioxidants that have many severe and harmful effects on human health.[7-9] In the desert environment of Egypt, MA fruits are used and are an important source of good nutrition because they contain many important nutrients such as polyphenolic compounds (radical scavenging properties), B-complex vitamins, fibers, amino acids, essential minerals (K, Na, Ca, and Mg), carbohydrates, glucose, and fatty acids.[7] In the Nubian desert in Egypt, fruits are used as a source of food for humans and mammals, where they are scarce.[9] The results of the research showed that exposure to gamma radiation significantly increased the DNA damage exemplified by the tail moment in the liver tissues. The DNA damage identified by cytogenetic analysis and the Comet assay can be produced from increased free oxygen radicals, resulting in a significant increase in harmful oxidative stress activity as an actual result of radioactive exposure.[1,2,36] Free oxygen radicals increased DNA damage and prevented cell division or disrupted DNA damage that induced mutations. The start of apoptosis results in the formation of a comet image; each comet image depicts DNA damage in a single cell and is composed of two major parts, namely the head and tail, which can be used as tools to detect radiation-induced DNA damage[37] On the other hand, rats that received an intraperitoneally administered injection of the ethanolic crude extract of MA (200 mg/kg b. wt.) significantly decreased the above parameters as compared to γ-radiated rats, indicating a positive therapeutic impact of MA in repairing the fragmented DNA due to exposure to γ-irradiation. Previous studies revealed that γ-radiation induced significantly increased ($P \leq 0.05$) liver functions ALT, AST, GGT, and ALP.[1,36] γ-irradiation increased the level of serum transaminases by increasing the oxidation of the liver cell membrane, which leads to destruction and increased permeability of the membrane, which eventually causes the transaminases to leak into the blood.[38] Elevated biochemical assays, including transaminases (AST, ALT), ALP, and GGT are known indicators of liver function and sensitive markers of confirmed liver injury and subsequent leakage of these enzymes from the tumor cell into the blood circulation in irradiated rats.[36] However, γ-radiation rats that were subsequently given MA (200 mg/kg b. wt.) for 6 weeks revealed a significantly decreased ($P \leq 0.05$) in liver functions (ALT, AST, GGT, ALP, and AFP); this is attributed to the increased synergistic activity of the powerful antioxidant polyphenolic compounds (radical scavenging properties) as well as other additional secondary metabolites.[7-9] γ-radiation induced a significantly increased ($P \leq 0.05$) in anti-inflammatory biomarkers (TNF-α, IL-6, CASP-3, MCP-1, and MMP-9), 8-OH-Dg, and DNA fragmentation.[2,36] However, γ-irradiation induced significantly decreased ($P \leq 0.05$) IL-10, total protein, and albumin.[36] γ-irradiation reduces inflammatory responses by inhibiting the release of inflammatory mediators (TNF-α, IL-6, CASP-3, MCP-1, and MMP-9).[36,39] Moreover, CASP-3 is a major and important enzyme in the apoptosis cascade, and it is often used to reveal the extent of apoptotic activity. MA treatment inhibited CASP-3 levels in rats.[9] The significant decrease in pro-inflammatory biomarkers, IL-6, TNF-α, and CASP-3 in γ-irradiated rats treated with MA ethanolic seed's extract is likely related to the potent anti-inflammatory and inhibitory effects of polyphenols in inhibiting nuclear factor-κB transmission and regulating inflammation-related expression of nitric oxide synthase. Unfortunately, the present investigation revealed that γ-irradiation treatment caused a significantly increased ($P \leq 0.05$) concentration of NO and MDA in parallel with a significantly decreased concentration of GSH, SOD, and CAT.[1,2,39-43] Gamma radiation increased the concentrations of NO and MDA. Radiolysis of water caused by gamma irradiation resulted in a significant decrease in antioxidant molecule levels.[44] The increased GGT activity of the γ-radiated group may be attributed to decreased hepatic GSH activity.[38] Preventing the high level of nitrogen oxide release during hepatocarcinogenesis in MA-treated animals reflects an improvement in the antioxidant status of the liver.[9] Antioxidant enzyme activity (GSH, SOD, and CAT) decreased significantly by γ-radiation which is distinctly capable of scavenging ROS.[1,39,43,45] Proanthocyanidins increase SOD activity.[43] Conversely, MA application at 200 mg/kg b. wt. for γ-irradiation treated rats effectively deceased NO and MDA concentrations significantly.[29] Furthermore, they significantly increased ($P \leq 0.05$) the activity of the antioxidant enzymes GSH, SOD, CAT, and POD.[29] In the desert environment of Egypt, MA fruits are used and are an important source of good nutrition because they contain many important nutrients such as polyphenolic compounds (radical scavenging properties), B-complex vitamins, fibers, amino acids, essential minerals (K, Na, Ca, and Mg), carbohydrates, glucose, and fatty acids, which are important for a good healthy diet to overcome oxidative stress caused by γ-radiation.[7-9] In the Nubian desert in Egypt, the fruits are used as a source of food for humans and mammals, where they are scarce.[9] HSP70 proteins are molecular chaperones that increase in response to stress and protect cells from oxidative or thermal stress.[46] HSP70 binds to its protein substrates and stabilizes them against aggregation or denaturation until conditions improve. HSP70 has many responsibilities during normal growth, including the degradation of unwanted proteins, the subcellular transport of proteins, and the folding of newly synthesized proteins.[47-49] γ-radiation-induced harmful oxidative stress, which increased HSP70 content as a biological response.[47] γ-radiation increased significantly ($P \leq 0.05$) the m-RNA transcript levels of HSP70 in the liver tissues of rats in comparison with control rats and rats treated only with MA. However, γ-irradiation treated rats that were subsequently given MA at 200 mg/kg b. wt. showed a significantly decreased ($P \leq 0.05$) level of HSP70 expression in liver tissues as compared to γ-irradiation treated rats, but still greater than normal. Because of its high antioxidant polyphenol content, MA extract has a high potential for lowering HSP70 levels in γ--irradiated rats. Malondialdehyde is important because it can also induce damage to DNA.[50] A highly active hydroxyl radical can break the DNA threads, creating 8-hydroxydeoxyguanosine. Moreover, the 8-OH-dG adduct was identified as a marker of DNA damage.[51] Exposure to gamma radiation leads to a significant dysfunction of the organs by oxidizing the lipid bilayer, thus affecting its integrity and greatly increasing its permeability.[7,39,52,53] Free radicals produced by γ-radiation alone caused lipid peroxidation, oxidative damage, and liver cell apoptosis in rat tissue, as evidenced by significantly increased liver MDA (cytotoxic aldehydes) content and significantly decreased total antioxidant activities.[39] MA administration to γ-irradiated rats decreased MDA concentration significantly ($P \leq 0.05$), indicating its powerful antioxidant polyphenols and numerous secondary metabolites.[7-9] Tissue can resist damage with the help of exogenous antioxidants.[40] The biochemical results agreed with the histopathological observations.

Conclusions

Our results showed that MA (a novel food) can be used for protecting patients and occupational workers during radiotherapy (in this area of research) as a natural therapeutic drug due to its valuable nutritional benefits, safe nature, and low cost. The lack of nutritional supplements in desert environments can be overcome using MA fruits. The great health-promoting potential of MA (potential prophylactic agent) against γ-radiation induced toxicity in rats may be related to the solidarity activity of its strong antioxidants, such as polyphenolic compounds (radical scavenging properties), B-complex vitamins, fiber, amino acids, essential minerals (K, Na, Ca, and Mg), carbohydrates, glucose, and fatty acids, which are important for a good healthy diet to overcome oxidative stress caused by γ-radiation. Natural antioxidant compounds present in MA are clearly capable of scavenging ROS and thereafter strongly improving the cellular balance (oxidant/antioxidant process), preserving normal liver functions, decreasing lipoperoxiation of cells, improving the immune defense systems, increasing antioxidant enzyme activities, and finally downregulating the signaling pathways responsible for the initiation of apoptotic proteins and pro-inflammatory cytokines.

Ethics code

The guidelines for the ethical use and maintenance of laboratory animals issued by the National Institutes of Health and authorized by the Scientific Committee of the Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt, were complied with in all procedures used in caring for rats and taking blood and tissue samples for this experiment.

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Conflicts of interest

There are no conflicts of interest.

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