

The Protective Potency of Medemia argun (An Egyptian Palm) Against Oxidative Stress, and Tissue Injury Induced by γ -Radiation in Rats

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Article

Keywords: Medemia argun, γ -irradiation, Oxidative stress, Radiotherapy, Inflammatory biomarkers

Posted Date: September 8th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1973451/v1>

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Abstract

Radiation damages living cells and affect all biological process in human body. Thereby, there is a magnificent interest for protecting patients from the aspect consequences radiotherapy and specialized professional workers by developing a natural antioxidant bio-drugs for amelioration of radiation hazards.

Medemia argun (an Egyptian Palm) is a mysterious from northern Sudan and southern Egypt (Nubian desert oases). In ancient Egypt, *Medemia argun* (MA) dried dates have found in the famed tomb of Tutankhamun. Globally, this is first study related to the metabolomics and biological efficiency of MA in alleviating the harmful effect of γ -irradiation.

This work highlights the ameliorative role of MA ethanolic seed's extract, as a natural promising functional food ingredient in alleviating radiation hazard via its antioxidative properties, anti-inflammatory, antiapoptotic and cell regeneration abilities. In this search, we have estimated the LD₅₀ of MA ethanolic seed's extract in vivo using male Wistar albino rats. The obtained results showed that 200 mg/kg b.wt. is the recommended dose.

Rats were randomly splitted into four groups. **Group I:** Represent as control (normal rats), that were received normal food and water daily for six weeks. **Group II.** Rats subjected to gamma radiation (6 Gy), a single dose of whole body after one 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 six weeks. **Group IV.** Rats subjected to gamma radiation (6 Gy), a single dose of whole body after one week of the experiment, then injected intraperitoneally with MA ethanolic seed's extract (200 mg/kg b.wt.) twice/week for six weeks. Each group contains 15 rats.

γ -radiation treatment caused a significant increase in DNA fragmentation, NO, MDA, inflammatory biomarkers (TNF- α , HsP70, IL-6, IL-10, 8-OH-dG, CASP-3, MPC-1, and MMP-9), and liver function (ALT, AST, GGT, ALP, and AFP). Also, there is a significant decrease in GSH, SOD, CAT, POD, 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. The potent therapeutic efficacy of MA was manifested in repairing the DNA fragmentation induced by γ -irradiation and this improvement confirmed by decrease in the concentration of 8-OH-dG.

To assess the biological activities and beneficial effect of the ethanolic extract of MA seed's in ameliorating the radiation hazards in rats, phytochemical analysis, were estimated which revealed presence of many beneficial natural health compounds such as, polyphenols (phenolics and flavonoids), proanthocyanidin (condensed tannins), saponins, protein and carbohydrate. Furthermore, histopathological examinations showed significant adverse deleterious changes in the structure of liver tissue due to exposure to γ -radiation, while treatment with MA maintains the cellular structure of the liver without the appearance of any changes. Biochemical data came to agree with the histopathological observations. There are no published reports of the radioprotective role of *Medemia argun* in rats.

Our results showed that MA can be used during radiotherapy as a natural therapeutic drug due to its valuable nutritional benefits, safe, nature and low cost.

Introduction

Exposure to gamma radiation destroys living cells, according to two theories, direct ionization of DNA and indirect theory via over production of reactive nitrogen species and reactive oxygen species which ends with cell death^{1,2}.

The hazard of γ -irradiation is due to its interaction with biological macromolecules such as proteins, lipids, and DNA in 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 radiation⁶. Gamma radiation excites the radiolysis of water in the entire cellular system, which induces increased production of reactive oxygen species (ROS), that causes a serious redox imbalance, which leads to an increase in oxidative stress in all cells and tissues⁷. Thereby, there is a great interest for protecting patients from the aspect consequences radiotherapy and specialized professional workers by developing a natural antioxidant bio-drugs for alleviation of radiation hazards. These bio-drugs are notably successful of scavenging ROS and in consequence improve the cellular/antioxidant balance, improving the immune defense system¹. The study highlights the protective role of MA as a promising nutritional and functional ingredient in hampering most of the harmful radiotherapy-induced outcomes via its nutritional benefits, antioxidative properties, anti-inflammatory, antiapoptotic and cell regeneration abilities^{7,8,9}. Such findings may offer an incentive in expanding its use during radiotherapy as a natural, safe, and low cost therapeutic drug.

Recently, scientists interest in searching for natural compounds as antioxidants from plant substances has increased due to ease of use, safe nature and low cost to avoid the use of artificial compounds that many poisonous facet effects and because of its very excessive costs².

In ancient times, the use of medicinal plants was a major source in treating human diseases. *Medemia argun* 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 of genus *Medemia*. The *Medemia argun* (MA) dried dates were found in the famed tomb of Tutankhamun in ancient Egypt^{10,11,12}. Until now, the importance of the presence of dried palm fruits in the tombs of the ancient Egyptians is still ambiguous. The fruits of MA are sweet, the flesh is thin and edible¹³. *Medemia argun* are 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^{7,8,9}.

A few studies have been conducted on the phytochemical analysis and biological activity of MA^{13,14}.

In the desert environment in Egypt, MA fruits are used and an important source of good nutrition because it contains 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 acids⁷. In the Nubian desert in Egypt, the fruits are used as a source of food for humans and mammals, where the fruits are scarce⁹.

Proanthocyanidin are class of polyphenolic compounds identified from MA nut and can be very useful as a protective agent against the harmful impacts of oxidative stress, which causes serious diseases (neurodegenerative, cancer, and cardiovascular diseases), antioxidant, antitumor, antimicrobial agents and against oxidative/nutritive destroy of blood platelet and plasma components^{8,9,13,15,16}.

Proanthocyanidin fraction from *Medemia* nuts has distinctive antioxidant properties and has a great ability to protect biomolecules (proteins, lipids, and DNA) that are exposed to increased level 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 (natural antioxidant) against reprotoxicity, tissue injury and oxidative stress caused by γ -radiation in rats.

Materials And Methods

Plant material.

In joint cooperation with researchers in botanical garden, fresh and healthy fruits of *Medemia argun* were obtained from Aswan University desert garden in October (2021) identified by Prof. SHEDED MG., according to¹⁰, and dried in dark at room temperature. The voucher specimens were saved in Faculty of Science, Botany Department Herbarium, Aswan University, Egypt.

Sample preparation and extraction of MA ethanolic seed's extract.

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

Metabolomic profiling and antioxidant of MA ethanolic seed's extract.

Polyphenols fractions (flavonoids and phenolic) were evaluated by HPLC (Waters, USA) by the method of ⁸. Proanthocyanidin was examined in the according to the methodology of ¹⁵. Saponins was identified by the procedure of ¹⁸. Crude protein content was measured the method of ⁷. Carbohydrate was measured by the method of ⁸.

Acute toxicity (LD₅₀) test for the MA ethanolic seed's extract.

Acute toxicity was identified by the method of ¹⁹. Briefly, 80 male healthy Wister albino rats (180-200 g) were treated intraperitoneally with *Medemia argun* 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 *Medemia argun* extract daily for one week. Rats were carefully under observation for any type of toxic symptoms and mortality for one week were recorded if they occurred. LD₅₀ dose was calculated on the basis of maximum (survival) and minimum (death) levels for each dose as follows:

$$LD_{50}: (M_0 + M_1)/2$$

Whereas, M₀ (maximum dose) of MA ethanolic seed's extract induced survival effects (no mortality) and M₁ (minimum dose) induced death (mortality) as shown in (Table1).

Source of Gamma-radiation.

The Gamma cell-40 irradiation unit (¹³⁷Cs), at 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 (single dose of whole body).

Experimental design, animal treatment and ethics potential.

Sixty adult male (13 weeks old), Wistar albino rats (n=15X4) weighing about 180-200 g were brought from Research Institute of Ophthalmology (animal house), Egypt, housed in cages (stainless steel) with good ventilated shields, the rats were acclimated with standard experiment conditions (26±2°C, 12/12 h light/dark, and 65% RH) for one week before the start of the experiment. During the whole experiment, all the animals were fed on 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 free access to drinking water *add libitum* for six weeks¹. Rats were randomly splited into four groups. **Group I:** Represent as control (normal rats) that were received normal food and water daily for six weeks. **Group II.** Rats subjected to gamma radiation (6 Gy), a single dose of whole body after one 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 six weeks. **Group IV.** Rats subjected to gamma radiation (6 Gy), a single dose of whole body after one week of the experiment, then injected intraperitoneally with MA ethanolic seed's extract (200 mg/kg b.wt.) twice/week for six weeks. Each group contains 15 rats.

The animals in this experiment were subjected to the recommendations for proper care of experimental animals²⁰, and specific local institutional laws 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 National Center for Radiation and Technology- Egyptian Atomic Energy Authority Cairo, Egypt (REC-NCRRT-34A/21). This research was done in compliance with the ARRIVE guidelines and regulations ([https:// arriveguidelines.org](https://arriveguidelines.org)).

Blood and tissue preparation.

Six weeks after the experiment was completed, rats were lightly anesthetized with isoflurane for blood sampling according to the ethics statement²¹ and blood is 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. Then, under deep anaesthesia all rats were sacrificed. 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 by using single cell gel electrophoresis analysis (*Comet* assay), that is simple technique, rapid, and sensitive to reveal the degree of DNA fragmentation (damage) by the method of^{22,23}.

Biochemical analysis

Serum investigation.

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), and alanine aminotransferase (ALT) activities, total protein and albumin concentrations were calculated using semi-automated analyzer (CCL-3001). Alpha-fetoprotein (α -fetoprotein) level was identified using rat AFP ELISA kit²⁴. Caspase-3 (CASP-3) levels and 8-hydroxy-deoxyguanosine (8-OHdG) were assessed by ELISA kit obtained from Life Sciences Advanced Technologies, in accordance with the manufacturer's requirements. The levels of tumor necrosis factor- α (TNF- α) was identified by Rat TNF-alpha using MyBioSource ELISA Kit (San Diego, CA, USA), Catalog no. MBS2507393. Metalloproteinase-9 (MMP-9) was assessed using ELISA kits (Rat MMP-9, Catalog no. E90553, China). Serum interleukin-10 (IL-10), monocytes chemoattractant protein-1 (MCP-1), and interleukin-6 (IL-6) were evaluated using MyBioSource ELISA Kit (San Diego, CA, USA), Catalog no. MBS2700945, MBS8804486, and MBS4500393, respectively. Total heat shock protein (HsP70) concentration were measured by ELISA Kit (Life Science Inc.). According to the manufacturer recommendations all measurements were performed.

Liver Tissue Investigations.

Nitric oxide was identified according to ²⁵. Malondialdehyde (MDA) level was measured by procedure of ²⁶. Superoxide dismutase (SOD) was calculated by the method of ²⁷. Reduced glutathione (GSH) were measured according to ²⁸. Catalase (CAT) activity was determined by the method of ²⁹.

Statistical analysis.

The data were presented as mean±SE. All the data were analyzed statistically according to the method of ³⁰ using SPSS statistical software package (v.17).

Results

Median Lethal Dose (LD₅₀) Experiment.

The estimated level LD₅₀ of MA ethanolic seed's extract was 400 mg/kg b.wt. So, half of this dose (200 mg/kg b.wt.) was considered the optimum dose (prophylactic dose) to be used during the experiment (Table1).

Table (1)

Estimation of LD₅₀ of the ethanolic crude extract of *Medemia argun* seed's.

Groups	Dose (mg/Kg b.wt.)	No. 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 ± SD of samples in triplicate.

The impact of prophylactic dose (200 mg/kg b.wt.) on the different molecular, biochemical parameters, and histological investigation was assessed (Table1).

Table (2)

Estimation of polyphenols (flavonoid and phenolic) estimated in the *Medemia argun* ethanolic seed's extract using HPLC

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

Values are mean $\pm$ SE of samples in triplicate.

To assess biological activities of *Medemia argun* ethanolic seed's extract, phytochemical and metabolomics profiling were assessed. Metabolomics profiling reported that twenty-two of polyphenols (phenolic and flavonoid fractions), were identified and their concentrations were evaluated using HPLC. as shown in Table (2). These polyphenols fraction have an important role in human health.

Table (3)

Quantitative analysis for the phytochemical constituents *Medemia argun* seed's

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

Values are mean ± SD of samples in triplicate.

Data as shown in Table (3) revealed the presence of many beneficial natural health compounds as saponins, protein, carbohydrate and proanthocyanidin (condensed tannins) identified in the *Medemia argun* ethanolic seed's extract.

Table (4)

Detection of genomic DNA damage in the different experimental rat groups

Parameters	Control	γ-irradiation (6 Gy)	MA (200 mg/Kg b.wt.)	MA + γ-irradiation (6 Gy)
Tailed %	4±0.09	15±0.45	3±0.12	7±0.42
Untailed %	96±3.84	85±4.8	97±5.32	93±6.51
Tail length (μm)	1.41±0.03	6.72±0.27	1.36±0.05	3.05±0.15
Tail moment (Unit)	2.17±0.06	47.91±1.62	2.04±0.06	9.66±0.67
Tail DNA (%)	1.54±0.04	7.13±0.36	1.50±0.04	3.17±0.13

Values are mean ± SE of samples in triplicate.

Determination of DNA damage by the *Comet* assay.

Comet assay is one of the most accurate technique for the determination of an adverse types of DNA damage. Using the Comet assay, to reveal liver genotoxic potential induced by gamma irradiation showed significantly increased level 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 injection of the ethanolic crude extract of *Medemia argun* (200 mg/kg b.wt.) significantly decreased the above parameters as compared to γ -irradiated rats indicating the positive therapeutic impact of *M. argun* in repairing the fragmented DNA due to exposure to γ -irradiation (Table 4 and Figure 1).

Histopathological assessment.

Liver tissues was fixed in 10% formol saline for twenty-four hours, for histopathological examination the liver specimens were fixed in buffered formaldehyde (10 %), slides are made by microtome (4 μ m thicknesses), and then immersed in paraffin wax. Sections were stained with hematoxylineosin (H&E), and examined using light microscope by method of ³¹.

In Figure (2): The photograph identified macroscopic manifestation for livers of rats in different groups. (a) Regular smooth surface liver appears in a control rat (b) γ -irradiated rats (hepatotoxic rat) describe a large zone of ductular cholangiocellular proliferation (arrow) implanted inside fibrosis and many irregular whitish micro- and macro-nodular on liver surface. (c) Normal smooth surface liver appears in a *Medemia argun* extract (hepatoprotective rat). (d) Nearly normal smooth surface liver of γ -irradiated+*Medemia argun* extract rats.

The effective role of *M. argun* in repairing the fragmented DNA and in alleviating the liver biochemical markers were confirmed by hepatic histopathological features. Hence, the examination of liver sections from γ -irradiation+*M. argun* treated rats showed a complete disappearance of sever histopathological changes induced by γ -irradiation (6 Gy) with few inflammatory cells diffusing between the hepatocytes (Figure 3).

In Figure (3) Histopathological sections of livers in different groups (a) Livers of the control rats appears normal architecture and histological structure (b) Livers of the γ -irradiated rats (hepatotoxic rat) showed the presence of pseudolobules with thick fibrotic septa and necrotic areas and acute structural damage (c) Livers of the *Medemia argun* extract (hepatoprotective rat) showed moderate inflammation without fibrotic septa (d) Livers of γ -irradiated+*Medemia argun* extract rats showed architecture with small zones of moderate necrosis and partially protected hepatocyte (Hematoxylin and Eosin stain original magnification: x20).

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 $\pm$ 1.31	75.3 $\pm$ 3.02	23 $\pm$ 0.69	37 $\pm$ 1.85
HsP70 (ng/ml)	7 $\pm$ 0.21	25 $\pm$ 1.25	5 $\pm$ 0.16	11 $\pm$ 0.77
IL-6 (pg/ml)	325 $\pm$ 19.5	573 $\pm$ 32.2	316 $\pm$ 15.7	390 $\pm$ 23.7
IL-10 (pg/ml)	408 $\pm$ 16.3	632 $\pm$ 37.9	398 $\pm$ 27.1	510 $\pm$ 30.6
CASP-3 (ng/100 mg tissue)	58 $\pm$ 3.2	171 $\pm$ 10.3	45 $\pm$ 2.7	73 $\pm$ 6.1
MPC-1 (pg/ml)	98 $\pm$ 5.1	302 $\pm$ 18.3	93 $\pm$ 2.8	145 $\pm$ 7.5
MMP-9 (ng/ml)	8 $\pm$ 0.42	31 $\pm$ 1.81	9 $\pm$ 0.62	13 $\pm$ 0.79
8-OH-dG (pg/mg tissue)	176 $\pm$ 10.7	289 $\pm$ 14.4	163 $\pm$ 12.9	197 $\pm$ 11.7
AFP (ng/ml)	5.13 $\pm$ 0.16	33.72 $\pm$ 2.02	4.73 $\pm$ 0.29	13.67 $\pm$ 1.09
ALT (U/l)	73 $\pm$ 0.9	164 $\pm$ 2.5	65 $\pm$ 4.7	92 $\pm$ 4.6
AST (U/l)	87 $\pm$ 4.3	195 $\pm$ 11.7	71 $\pm$ 2.8	108 $\pm$ 9.6
GGT (U/l)	36 $\pm$ 1.8	98 $\pm$ 5.9	29 $\pm$ 1.7	48 $\pm$ 3.3
ALP (U/l)	97 $\pm$ 5.72	236 $\pm$ 18.5	85 $\pm$ 5.3	120 $\pm$ 7.1
Albumin (g/dl)	4.73 $\pm$ 0.44	2.35 $\pm$ 0.15	4.53 $\pm$ 0.19	4.18 $\pm$ 0.35
T. protein (g/dl)	6.41 $\pm$ 0.39	3.98 $\pm$ 0.17	6.58 $\pm$ 0.33	5.27 $\pm$ 0.28
MDA (nM/g tissue)	12.9 $\pm$ 0.78	29.7 $\pm$ 1.80	11.8 $\pm$ 0.84	18.6 $\pm$ 1.14
NO (μ M/ml)	0.97 $\pm$ 0.12	8.15 $\pm$ 0.36	0.69 $\pm$ 0.04	3.08 $\pm$ 0.27
SOD (U/g tissue)	98 $\pm$ 4.9	52 $\pm$ 3.1	102 $\pm$ 9.1	84 $\pm$ 5.1
GSH (U/g tissue)	128 $\pm$ 8.9	84 $\pm$ 7.2	112 $\pm$ 6.6	99 $\pm$ 5.6
CAT (U/g tissue)	85 $\pm$ 5.3	52 $\pm$ 3.1	88 $\pm$ 6.1	71 $\pm$ 4.1

Values are mean $\pm$ SE of samples in triplicate.

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, MPC-1, and MMP-9), in comparison with the control. While, there is a significantly decline ($p \leq 0.05$), in GSH, CAT, SOD, T. protein, 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 significantly increment ($p \leq 0.05$), in m-RNA transcript levels of heat shock protein (HsP70) in liver tissues of rats in comparison with control 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 Table (4 and 5) and Figure (1). The potent therapeutic efficacy of MA was manifested in repairing the DNA fragmentation induced by γ -irradiation and this improvement confirmed by decrease in the concentration of 8-OH-dG Table (4 and 5) and Figure (1).

Our data proven a prophylactic potential of MA ethanolic seed's extract on γ -irradiation- tolerate rats at many sides as shown previously.

Discussion

Radiolysis induced by gamma radiation generates a lot of ROS which eventually severely damage living cells and cause 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 that leads to high damage and destruction of living tissues⁹. In recent years, many antioxidants have been used for their supposed or actually beneficial effect against oxidative stress, such as polyphenols, saponins, and vitamin E³³.

Recently, scientists interest in searching for natural compounds as antioxidants from plant substances has increased due to ease of use, safe nature and low cost to avoid the use of artificial compounds that many poisonous facet effects and because of its very excessive costs^{2,8}.

In the Nubian Desert in southern Egypt, and northern Sudan a rare type of fan palm (*Medemia argun*) is found^{10,11,34}, and 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 proinflammatory mediator cytokines and health-damaging programmed proteins^{7,8,9}.

The median lethal dose (LD₅₀) for *Medemia argun* ethanolic seed's extract (200 mg/Kg b.wt.) was similar to that obtained by³⁵.

In this study, polar solvents, such as ethanol, were used because it is high efficient than other non-polar solvents and water in extracting antioxidants compounds from MA. Twenty-two fractions of polyphenols (phenolic and flavonoid). *Medemia argun* contains a range of other bioactive secondary metabolites with

important, beneficial and antioxidant properties such as, saponins, protein, proanthocyanidin (condensed tannins), and carbohydrate, which are similar to that of ^{7,8,9}.

Medemia argun rich with proanthocyanidins that contains subunits (catechin, afzelechin, and galocatechin). The high therapeutic value of MA may be related to 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, neurodegeneration, cardiovascular and cancer¹⁵.

Antioxidant polyphenols (radical scavenging properties) in MA, which is characterized as being natural and has many health benefits, which can be recommended for use in food industry (as important and useful nutritional supplements) instead of artificial antioxidants that have many severe and harmful effects on human health^{7,8,9}.

In the desert environment in Egypt, MA fruits are used and an important source of good nutrition because it contains 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 (Mohammed *et al.*, 2019). In the Nubian desert in Egypt, the fruits are used as a source of food for humans and mammals, where the fruits are scarce ⁹.

The results of the research showed that exposure to gamma radiation increased significantly DNA damage exemplify by the tail moment in the liver tissues. The DNA damage identified in cytogenetic analysis and Comet assay can be produced from increased free oxygen radicals and thus 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 prevent cell division (unrepaired) or disrupted damage that induced mutations. Starting of apoptosis produce Comet picture, that values and tail parameters are similar to cells with mild DNA damage ³⁷. On the other hand, rats that received an intraperitoneally injection of the ethanolic crude extract of *Medemia argun* (200 mg/kg b.wt.) significantly decreased the above parameters as compared to γ -radiated rats indicating positive therapeutic impact of *M. argun* in repairing the fragmented DNA due to exposure to γ -irradiation.

Previous study revealed that γ -radiation induced a significantly increased ($p \leq 0.05$) liver functions, ALT, AST, GGT, AFP and ALP ^{1,36}. γ -irradiation increased level of serum transaminases by increasing the oxidation of the liver cell membrane, which leads to destruction and increased permeability of 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 in irradiated rats from the tumor cell into the blood circulation ³⁶.

However, γ -radiation rats that were subsequently given MA (200 mg/kg b.wt.) for six weeks revealed significantly decrease ($p \leq 0.05$) 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^{7,8,9}.

γ -radiation induced a significantly increase ($p \leq 0.05$) in anti-inflammatory biomarkers (TNF- α , IL-6, CASP-3, MPC-1, and MMP-9), 8-OH-Dg and DNA fragmentation^{2,36}. However, γ -irradiation induced a significantly decreased ($p \leq 0.05$) in IL-10, total protein and albumin³⁶.

γ -irradiation inhibit the release of the inflammatory mediators (TNF- α , IL-6, CASP-3, MPC-1, and MMP-9) for decreasing inflammatory responses^{36,39}.

Moreover, caspase-3 is a major and important enzyme in the apoptosis cascade and it is very often to reveal the extent of apoptotic activity. MA treatment inhibited caspase-3 levels in rats⁹.

Decreasing significantly of 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- κ B transmission and regulating inflammation-related expression of nitric oxide synthase¹.

Unfortunately, the present investigation revealed γ -irradiation treatment caused significantly increase ($p \leq 0.05$) in concentration of NO, and MDA in parallel with significantly decrease in GSH, SOD, and CAT^{1,2,39,40,41,42,43}. Gamma radiation increased concentration of (NO, and MDA). Radiolysis of water induced by gamma irradiation caused a marked decrease in the levels of antioxidant molecules ⁴⁴. Increased GGT activity of γ -radiated group may be attributed to the decreased hepatic GSH activity³⁸.

Preventing the high level of nitrogen oxide release during hepatocarcinogenesis of MA-treated animals reflects an improvement in the antioxidant status of the liver⁹.

Antioxidant enzyme activity (GSH, SOD, and CAT) decreased significantly by γ -radiation which are distinctly capable of scavenging ROS^{1,39,43,45}. Proanthocyanidin increased significantly SOD activity⁴³.

Conversely, MA application at 200 mg/kg b.wt. for γ -irradiation treated rats effectively decreased significantly ($p \leq 0.05$) NO, and MDA concentrations²⁹. Also, they significantly increased ($p \leq 0.05$) activity of antioxidant enzyme GSH, SOD, CAT, and POD ²⁹.

In the desert environment in Egypt, MA fruits are used and an important source of good nutrition because it contains 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 is important for a good healthy diet to overcome oxidative stress caused by γ -radiation^{7,8,9}. In the Nubian desert in Egypt, the fruits are used as a source of food for humans and mammals, where the fruits are scarce⁹.

Hsp70 proteins is a molecular chaperone which increased in response to stress and protect cells from oxidative or thermal stress⁴⁶. Hsp70 binds to its protein substrates and stabilize them against aggregation or denaturation until conditions improve. Hsp70 has many responsibilities during normal growth; degradation of unwanted proteins, subcellular transport of proteins, and helps in the folding of newly synthesized proteins^{47,48,49}. γ -radiation-induced harmful oxidative stress, which increased HsP70 content as a biological response⁴⁷.

γ -radiation increased significantly ($p \leq 0.05$), m-RNA transcript levels of heat shock protein (HsP70) in liver tissues of rats in comparison with control 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, but still greater than normal. Our result points the great potential impact of MA extract, due to its high antioxidant polyphenols contents, in decreasing the HsP70 level in γ -irradiated rats.

Malondialdehyde, are important because they can also induce damage DNA⁵⁰. 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 damage⁵¹.

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 generated by γ -radiation alone induced lipid peroxidation, oxidative damage and liver cell apoptosis in rat tissue as proven by significantly increase in liver MDA (cytotoxic aldehydes) content; and significant decrease in total antioxidant activities³⁹.

MA administration to γ -irradiated rats significantly ($p \leq 0.05$) decreased the MDA concentration, indicating its powerful antioxidant polyphenols and many secondary metabolites^{7,8,9}. With the help of exogenous antioxidants, tissues can resist damage⁴⁰. Biochemical results came to agree with the histopathological observations.

Conclusion

Our results showed that MA (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 by 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 high strong antioxidant 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 is 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), preserve normal liver functions and decreasing lipoperoxidation of cells, improving the immune defense systems, increasing antioxidant enzymes activities and finally downregulating the signaling pathways responsible for initiation of apoptotic proteins and proinflammatory cytokines.

Declarations

Statement of ethics. 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.

Author contributions

All authors conceived and designed the experiment performed all the in vivo experiments. Moussa, H.R., Moataz Ahmed Naguib Abd-Allah and Mohamed Ebrahim Abdel-alim performed the in vivo experiments and data management and statistics. Fathy A. El-Saied and Nesrein Saad Salim performed all the in vitro assays and all authors contributed in writing and reviewing the manuscript.

Conflict of Interest

Authors declare that no conflict of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgements

“Not Applicable”

Competing interests The authors declare no competing interests.

Plant material All methods were performed in accordance with regulations and guidelines.

Financial Support and Sponsorship This research did not receive any specific grant from funding bodies in the public, commercial, or not-for profit sectors.

Availability of data and materials All data generated or analyzed during this study are included in this published article.

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Figures

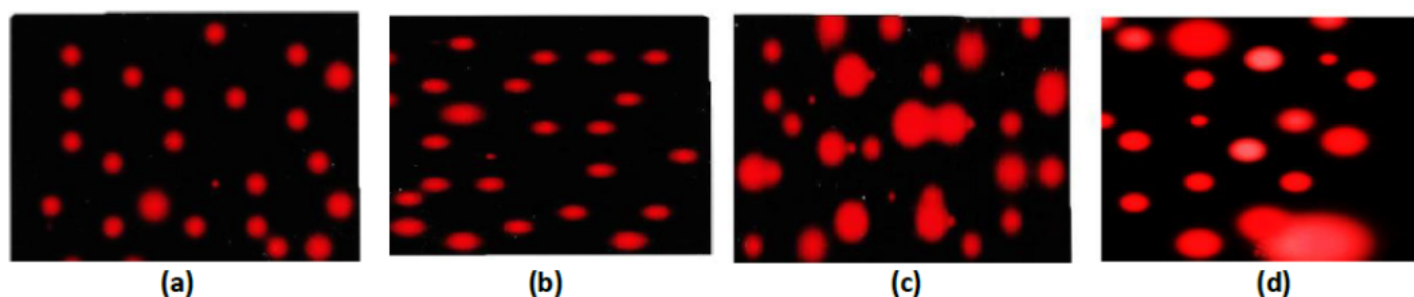


Figure 1

Photograph showing identification of DNA fragmentation of male rat liver using Cometassay in the experimental groups, (a) Control, (b) *Medemia argun* extract, (c) γ -irradiation (6 Gy), (d) γ -irradiation (6 Gy) + *Medemia argun* extract.

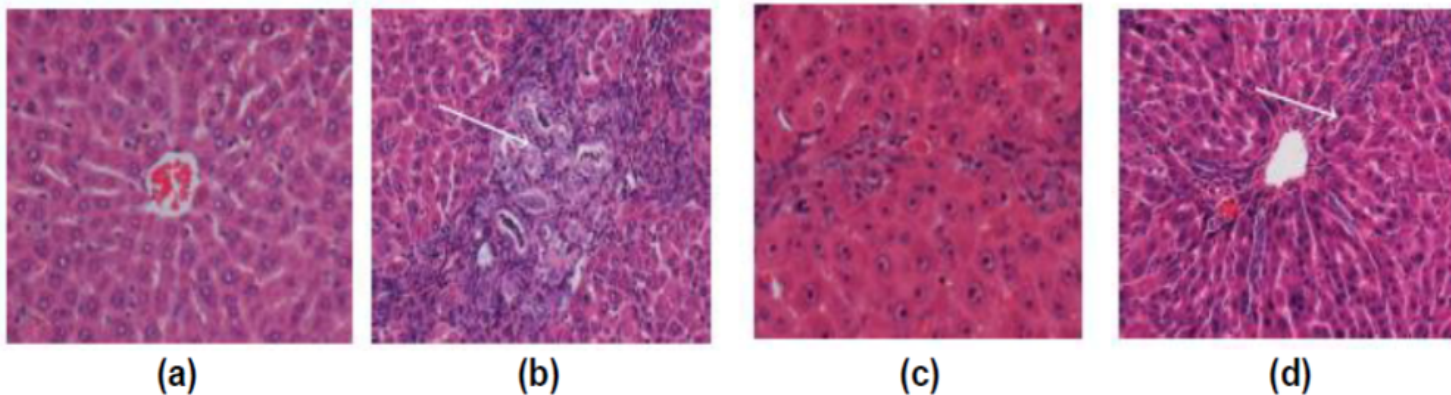


Figure 2

Photograph of morphological livers in different groups, (a) Control, (b) γ -irradiation (6 Gy), (c) *Medemia argun* extract, (d) γ -irradiation (6 Gy) + *Medemia argun* extract.

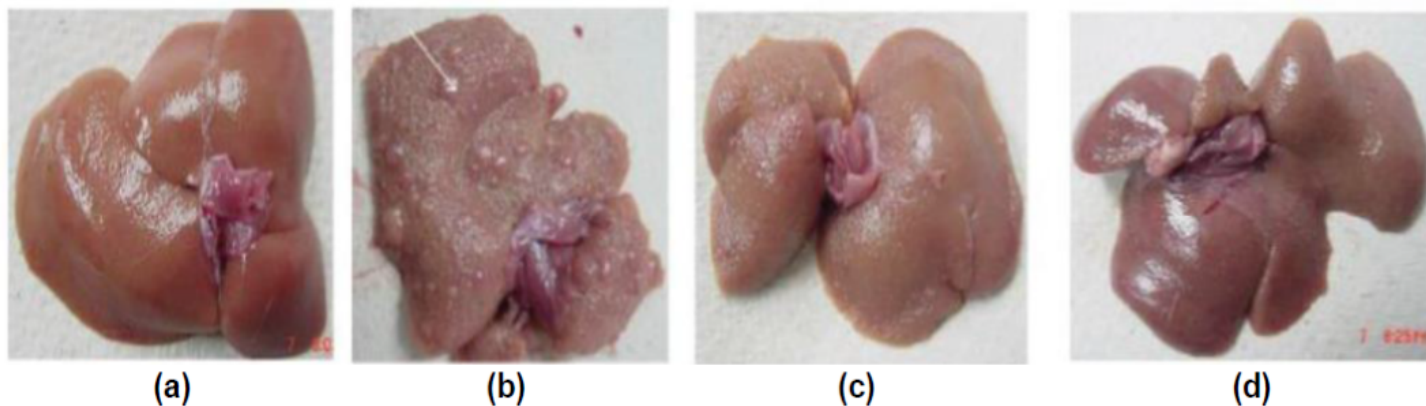


Figure 3

Histopathological segment of livers in different groups, (a) Control, (b) γ -irradiation (6 Gy), (c) *Medemia argun* extract, (d) γ -irradiation (6 Gy) + *Medemia argun* extract.