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Nephro Radioprotective Potential of Eucalyptus Oil in γ-Irradiated Rats

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Abstract

Background: Eucalyptus oil (EO) are used for medicinal purposes. The radio-protective activity of EO was investigated against γ-rays-induced nephrotoxicity in rats. The radio protective potential of EO was investigated in the kidney of the γ-irradiated rats.

Methods: Thirty-two rats were equally divided into 4 groups; control, EO (100mg/kg), irradiated (6Gy γ-rays, single dose) and EO plus γ-rays treated groups. The serum creatinine (CRT) and asymmetric dimethylarginines (ADMA) were estimated. The renal tissues lipid peroxidation; malondialdehyde (MDA), total nitrate/nitrite (NO(x)), superoxide dismutase (SOD), reduced glutathione (GSH), levels, glutathione peroxidase (GSHPx), tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), have been studied.

Results: A significant increase in the levels of serum content of CRT and ADMA in the γ-rays group. The results also showed significantly increased level of renal MDA, NO(x), and the inflammatory markers, and decreased levels of renal antioxidants markers in γ-rays treated groups. All these indices results were significantly improved in the EO plus γ-rays treated groups.

Conclusion: EO prevents γ-rays-induced renal damage that could attribute to its antioxidant, anti-inflammatory and radio-protective activities.

Introduction

Ionized radiation (≥6Gy γ-rays)-induced cytotoxic effects and hazardous effects in kidney [1-3]. In a recent study, the authors concluded that gamma-rays (7.5 Gy) prompted a significant raise of renal biochemical indices: creatinine, oxidative-stress indicators, MDA concomitant with a reduction of GSH and SOD. Beside a raise in the level of the renal inflammatory mediators: TNF-α, and IL-1β [4]. In an endeavour to minimise these cytotoxic effects, antioxidant compounds have been identified to counteract radiation-associated toxicities [5,6].

The genus Eucalyptus L'Heritier comprises about 900 species, over 300 of them contain volatile essential oil in their leaves. EO has amazing widespread biological activities including antimicrobial, antiseptic, antioxidant, chemotherapy agent, liver and gastrointestinal disorders treatment and wound healing [7]. Many study evaluate the beneficial effects of EO upon oxidative stress-induced kidney damages in rat [8,9] and in mice [10].

We have made an attempt to evaluate the biological role of EO, as a natural medicine for mitigation of radiation-induced tissue injury. Biochemical assess in serum and renal tissues were performed.

Material and methods

Animals

Male albino rats (250–270g) were obtained from the Egyptian organization for biological product and vaccines Giza, Egypt. Animal were acclimated weeks before experimentation and were received standard diet and water ad libitum and maintained under standard conditions of humidity, temperature (20-24°C), and 12-h light-dark cycle. Animals were deprived of food, but not water, overnight before experiments. All experiments were performed in accordance with ethics committee of NCRRT.

Radiation processing

It was performed by using gamma cell-40 (cesium-137) located at NCRRT, Cairo, Egypt. Animals were irradiated with a single dose level of 6 Gy γ-rays, delivered at a dose rate of 0.42Gy/ min at the time of experimentation. Animals were not anesthetized before irradiation.

Chemicals

Pure Eucalyptus oil (100%) was used, extracted by steam distilled from leaves and small branches, from (NOW Eucalyptus Essential Oil-NOW Foods, Bloomingdale, USA). All other chemicals and solvents used were of the highest purity grade available.

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Table 1. The levels of the inflammatory markers in serum of different rat groups

PARAMETERS	GROUPS			
	Control	EO	Irradiated	EO + γ-rays
CRT (mg/ dl)	0.72± 0.042	0.74± 0.031	2.68± 0.105[a]	1.12± 0.054[b]
ADMA (umol/ l)	0.46± 0.032	0.47± 0.041	1.12± 1.114[a]	0.58± 0.072[b]

All values are expressed as mean± S. E.
[a]Significant difference from control group.
[b]Significant difference from γ-rays group.

Experimental design

Animal grouping: Rats were randomly distributed into four groups, each consisting of 8 rats. They were control group: animals received normal saline (N/S); 2 ml/ kg body weight as a vehicle for 7 successive days, intra gastric (ig). EO group: animals received EO (100 mg/ kg body weight, ig) for the same time, according to Mansour [11]. Irradiated group: animals received N/S with the same dose and time; then exposed to a single dose of (6 Gy γ-rays). EO + γ-rays group: EO with the same dose and time was given prior to irradiation. Examination was carried out 7 days after end of the experiment. The animals were anesthetized, sacrificed and their kidneys were dissected.

Biochemical analysis: 24 hours after the last dose of the specific treatment, blood samples were obtained by heart puncture and serum was separated. Blood serum was prepared for measuring creatinine (CRT) using colorimetric kits (Abcam, UK) according to the manufacturer's instructions. The absorbance was read at 570nm. Asymmetric dimethylarginines (ADMA) was estimated using a standard enzyme linked immunosorbent assay (ELISA) method according to the manufacturer's instructions (Immundiagnostik AG, Bensheim/Germany).

Kidneys were quickly removed, washed with saline, blotted with a piece of filter paper. Kidney homogenates were prepared for estimation of oxidative stress markers; malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) levels and measured using commercial kits (Zellbio GmbH, Germany) according to the manufacturer's instructions. The absorbance was read at 420m, 412nm, 535nm and 412nm, respectively. Also, kidney homogenates were used for measuring total nitrate/nitrite (NO(x)) level using colorimetric kits (Abcam, UK) according to the manufacturer's instructions. The absorbance was read at 540nm.

Detection of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the renal's homogenate in the different rat groups were executed by ELISA technique (BioSourcs International, Camarillo, USA), according to the company's directions. Each sample assess was frequent three times.

Statistical analysis: Data were analysed using one-way analysis of variance (ANOVA) followed by LSD post hoc test. The results obtained were expressed by mean± standard deviation. Differences were considered significant at $p \leq 0.05$ [12].

Results

As presented in table 1-4, animal group treated with EO only showed non-significant changes in all estimated parameters compare to control group.

A significant increase in the levels of serum content of creatinine and ADMA in irradiated group as compared to control rat group (Table 1). Administration of an ig EO (100 mg/ kg body weight) for 7 repeated days before 6 Gy γ-rays-exposure significantly decreased the levels of CRT and ADMA in serum ($p \leq 0.05$) compared to the EO + γ-rays group (Table 1).

Also, the effects of γ-rays on oxidative stress markers are shown in Table 2. The γ-rays induced a significant augmentation in MDA and NO(x) contents and significant diminution in the renal SOD and GSHPx activities and GSH content, compared with control group. Administration of EO prior to γ-rays-exposure resulted in a significant reduction in MDA and NO(x) levels and significant increases in the activity of renal SOD and GSHPx, and content of GSH compared to the EO + γ-rays group (Table 2).

As shown in Table 3, significant augmentation in some inflammatory markers represented in renal tissues; TNF-α and IL-1β levels were observed in γ-irradiated group compared with corresponding values of control group. The administration of EO before exposure to γ-rays significantly limited the elevation in those inflammatory markers levels compared to irradiated group (Table 3).

Discussion

The present study aimed to investigate the potential protective effect of EO against γ-rays-induced renal toxicity in rats. In addition, the possible mechanisms underlying the renal protective effect were explored including the antioxidant as well as the anti-inflammatory effects. Cellular exposure to ionising-radiation leads to oxidative stress events, which refer to raised intracellular levels of reactive oxygen species (ROS). The elevated levels of ROS significantly contributed to γ-radiation induced cytotoxicity [5]. In the present study, 6 Gy γ-rays caused a marked increase in serum levels of creatinine and ADMA. These data agree with that reported in previous studies, which reported that γ-irradiation caused a significant increase in creatinine and ADMA [11,13]. The pre-treatment with EO for 7 successive days prior to irradiation ameliorated the level of serum creatinine and ADMA. This effect might be related to the anti-oxidative properties of EO,

Table 2. The levels of oxidative stress markers in renal tissues of different rat groups

PARAMETERS	GROUPS			
	Control	EO	Irradiated	EO + γ-rays
MDA (nmol/ g tissue)	145.8± 2.34	146.2± 2.64	241.6± 6.44[a]	1.71± 9.23[b]
NO(X) (µmol/ g tissue)	27.5± 0.66	27.4± 0.73	56.6± 1.67[a]	33.8± 1.45[b]
SOD (µg/ g tissue)	95.4± 1.35	94.7± 1.12	45.6± 0.86[a]	74.22± 1.01[b]
GSH (µmol/ g tissue)	0.22±0.002	0.22±0.001	0.11± 0.001[a]	018± 0.001[b]
GSHPX (mole/ g tissue)	0.43±0.003	0.43±0.002	0.35± 0.011	0.42± 0.002

All values are expressed as mean± S. E.
[a]Significant difference from control group.
[b]Significant difference from γ-rays group.

Table 3. The levels of the inflammatory markers in renal tissues of different rat groups

PARAMETERS	GROUPS			
	Control	EO	Irradiated	EO + γ-rays
TNF-A (pg/ ml)	33.43± 0.97	34.01± 0.81	69.11± 2.03[a]	36.68± 1.51[b]
IL-1B (pg/ ml)	15.97± 1.27	15.22± 1.24	44.12± 1.23[a]	18.56± 1.42[b]

All values are expressed as mean± S. E.
[a]Significant difference from control group.
[b]Significant difference from γ-rays group.

which protect against oxidative stress, wherever EO showed potential cyto-protective and anti-oxidative property against oxidative stress in cells [14].

Consistent with some previous studies [15,16], the present study showed a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxides; MDA and NO(x) levels in renal tissues after whole body γ-rays-irradiation.

Ionizing radiation is known to induce oxidative stress through generation of ROS in an imbalance in pro-oxidant, antioxidant status in the cells and lead to cellular damage (e.g. lipid peroxidation) and cell death [17]. The increase in MDA level in γ-irradiated rats might be due to the interaction of free radicals with polyunsaturated fatty acids in the phospholipids portion of cellular membranes [18].

The current results showed that whole body γ-irradiation of rats at 6 Gy enhanced the formation of renal NO(x). Similar results have been reported by Abu-Khudir et al. [19]. Gamma-irradiation may enhance endogenous NO-biosynthesis in animal tissues, presumably by facilitating the entry of Ca^{2+} ions into the membrane as well as the cytosol of NO-producing cells through irradiation-induced membrane lesions. The enhancement of NO-production following exposure to γ-rays was attributed to high levels of expression of the inducible NO-synthase [20].

The decrease in the activities of SOD and GSHPx and the decreased level of GSH might be due to their utilization by the enhanced production of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation [21].

GSH, a well-known antioxidant, provides major protection in oxidative injury by participating in the cellular system of defense against oxidative damage. Tissue GSH levels and the activities of glutathione reductase and GSHPx are critical constituents of GSH needed for antioxidant protection, were significantly reduced due to oxidative stress, permitting enhanced free radical-induced tissue damage [22].

The TNF-α and IL-1β show a protagonist in initiating the inflammatory responses [23]. It was concluded that the desperate exposure to γ-rays enhanced the excretion of inflammatory cytokine markers, which is in agreement with the findings of a previous study by Elkady and Tawfik [24]. In the present work, EO revealed anti-inflammatory properties via inhibiting significantly the γ-rays-induced improved level of pro inflammatory markers (TNF-α and IL-1β). The decrease of IL-1β expression in EO + γ-rays group showed that EO could control inflammation. The EO reduced inflammatory (TNF-α and IL-1β) marker levels caused by sepsis and ameliorated lipopolysaccharide-induced endothelial cell injury in human umbilical-cord vein endothelial-cells [25].

Pre-treatment with EO, at a dose of 100 mg/kg, significantly ameliorated γ-rays-induced oxidative stress, increased GSH, SOD and GSHPx till reached to normal values in parallel with a significant decrease in ADMA, MDA and NO(x) levels, which confirmed the strong antioxidant effect of EO [7-9]. Experimental evidence indicated that antioxidant compounds can be used to protect against radiation-induced renal toxicity [26]. In addition, Chen et al. [27] found that EO exhibited great antioxidant activity in both chemical- and cellular-based antioxidant assays and suggested that it would be promising natural antioxidants in pharmaceutical and industries. Moreover, in human and animal health, EO has good antioxidant and anti-inflammatory activities on the liver and kidney [28] and greatly improved the antioxidant property *in vitro* [29].

The results from the present investigation indicate that EO pre-treatment protects against radiation damage by inhibiting radiation-induced oxidative stress by decreasing CRT, ADMA, MDA, NO(x) levels and ameliorating the antioxidant system and the inflammatory markers the in renal tissues.

The current study is the first in the literature that EO had radio protective effects on kidneys, in terms of biochemistry and tissue inflammation when administered concomitant with radiation exposure.

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Consent to participate

The study was approved by Central Scientific Publishing Committee, Egyptian Atomic Energy Authority, No. 199 at November 2020.

References

1. Aziz MM, Eid NI, Nada AS, Amin NE, Ain-Shoka AA (2018) Possible protective effect of the algae spirulina against nephrotoxicity induced by cyclosporine A and/or gamma radiation in rats. *Environ Sci Pollut Res Int* 25: 9060-9070. [Crossref]

2. Ma N, Kato T, Isogai T, Gu Y, Yamashita T (2019) The potential effects of taurine in mitigation of radiation nephropathy. *Adv Exp Med Biol* 1155: 497-505.

3. Azzam P, Francis M, Youssef T, Mroueh M, Daher AA, et al. (2021) Crosstalk between SMPDL3b and NADPH oxidases mediates radiation-induced damage of renal podocytes. *Front Med (Lausanne)* 8: 732528. [Crossref]

4. Hasan HF, Elgazzar EM, Mostafa DM (2020) Diminazene aceturate extenuate the renal deleterious consequences of angiotensin-II induced by γ-irradiation through boosting ACE2 signaling cascade. *Life Sci* 253: 117749.

5. Salem AA, Ismail AFM (2021) Protective impact of Spirulina platensis against γ-irradiation and thioacetamide-induced nephrotoxicity in rats mediated by regulation of micro-RNA 1 and micro-RNA 146a. *Toxicol Res (Camb)* 10: 453-466.

6. Mahgoub S, Sallam AO, Sarhan HKA, Ammar AAA, Soror SH (2020) Role of Diosmin in protection against the oxidative stress induced damage by gamma-radiation in Wistar albino rats. *Regul Toxicol Pharmacol* 113: 104622.

7. Dhakad AK, Pandey VV, Beg S, Rawat JM, Singh A (2018) Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: a review. *J Sci Food Agric* 98: 833-848.

8. Dhibi S, Mbarki S, Elfeki A, Hfaiedh N (2014) Eucalyptus globulus extract protects upon acetaminophen-induced kidneydamages in male rat. *Bosn J Basic Anti-inflammatory Med Sci* 4: 99-104.

9. Hu Z, Feng R, Xiang F, Song X, Yin Z, et al. (2014) Acute and subchronic toxicity as well as evaluation of safety pharmacology ofeucalyptus oil-water emulsions. *Int J Clin Exp Med* 7: 4835-4845. [Crossref]

10. Ghareeb MA, Sobeh M, El-Maadawy WH, Mohammed HSh, Khalil H, et al. (2019) Chemical profiling of polyphenolics in Eucalyptus globulus and evaluation of its hepato-renal protective potential against cyclophosphamide induced toxicity in mice. *Antioxidants (Basel)* 8: 415.

11. Mansour HH (2013) Protective effect of ginseng against gamma-irradiation-induced oxidative stress and endothelial dysfunction in rats. *EXCLI J* 12: 766-777.

12. Snedecor GW, Cochran WG (1994) Statistical Methods, 8th Edition, Calcutta, India: Oxford & IBH Publishing Co, p. 503.

13. Saberi H, Keshavarzi B, Shirpoor A, Gharalari FH, Rasmi Y (2017) Rescue effects of ginger extract on dose dependent radiation-induced histological and biochemical changes in the kidneys of male Wistar rats. *Biomed Pharmacother* 94: 569-576.

14. Ganesan D, Al-Sayed E, Albert A (2018) Antioxidant activity of phenolic compounds from extracts of Eucalyptus globulus and Melaleuca styphelioides and their protective role on D-glucose-induced hyperglycemic stress and oxalate stress in NRK-49Fcells. *Nat Prod Res* 32: 1274-1280. [Crossref]

15. Kuzay D, Ozer C, Sirav B, Canseven AG, Seyhan N (2017) Oxidative effects of extremely low frequency magnetic field and radio frequency radiation on testes tissues of diabetic and healthy rats. *Bratisl Lek Listy* 118: 278-282.

16. Mercantepe T, Topcu A, Rakici S, Tumkaya L, Yilmaz A (2019) The radioprotective effect of N-acetylcysteine against x-radiation-induced renal injury in rats. *Environ Sci Pollut Res Int* 26: 29085-29094.

17. de Jager TL, Cockrell AE, Du Plessis SS (2017) Ultraviolet light induced generation of reactive oxygen species. *Adv Exp Med Biol* 996: 15-23.

18. Guéraud F, Taché S, Steghens JP, Milkovic L, Borovic-Sunjic S, et al. (2015) Dietary polyunsaturated fatty acids and heme iron induce oxidative stress biomarkers and a cancer promoting environment in the colon of rats. *Free Radic Biol Med* 83: 192-200.

19. Abu-Khudir R, Habieb ME, Mohamed MA, Hawas AM, Mohamed TM (2017) Anti-apoptotic role of spermine against lead and/or gamma irradiation-induced hepatotoxicity in male rats. *Environ Sci Pollut Res Int* 24: 24272-24283.

20. Fardid R, Salajegheh A, Mosleh-Shirazi MA, Sharifzadeh S, Okhovat MA, et al. (2017) Melatonin ameliorates the production of COX-2, iNOS, and the formation of 8-OHdG in non-targeted lung tissue after pelvic irradiation. *Cell J* 19: 324-331.

21. Hadj Abdallah N, Baulies A, Bouhlel A, Bejaoui M, Zaouali MA, et al. (2018) Zinc mitigates renal ischemia-reperfusion injury in rats by modulating oxidative stress, endoplasmic reticulum stress, and autophagy. *J Cell Physiol* 233: 8677-8690. [Crossref]

22. Dubinsky JM (2017) Towards an understanding of energy impairment in Huntington's disease brain. *J Huntingtons Dis* 6: 267-302.

23. Presta P, Bolignano D, Coppolino G, Serraino F, Mastroroberto P, et al. (2021) Antecedent ACE-inhibition, inflammatory response, and cardiac surgery associated acute kidney injury. *Rev Cardiovasc Med* 22: 207-213.

24. Elkady AA, Tawfik SS (2018) Role of piperine against rat lung tissue damage induced by gamma-rays. *Int J Radiat Res* 16: 75-84.

25. Kutlu Z, Gulaboglu M, Halıcı Z, Cınar İ, Dıyarbakır B (2021) Biochemical research of the effects of essential oil obtained from the fruit of Myrtus communis L. on cell damage associated with lipopolysaccharide-induced endotoxemia in a human umbilical cord vein endothelial cells. *Biochem Genet* 59: 315-334. [Crossref]

26. Mahmoud Moustafa E, Rashed ER, Rashed RR, Omar NN (2021) Piceatannol promotes hepatic and renal AMPK/SIRT1/PGC-1α mitochondrial pathway in rats exposed to reserpine or gamma-radiation. *Int J Immunopathol Pharmacol* 35: 20587384211016194. [Crossref]

27. Chen Y, Wang J, Ou Y, Chen H, Xiao S, et al. (2014) Cellular antioxidant activities of polyphenols isolated from Eucalyptus leaves (Eucalyptus grandis×Eucalyptus urophylla GL9). *J Funct Foods* 7: 737-745.

28. Zhao C, Cao Y, Zhang Z, Nie D, Li Y (2021) Cinnamon and eucalyptus oils suppress the inflammation induced by lipopolysaccharide in vivo. *Molecules* 26: 7410. [Crossref]

29. Chen M, Yan X, Cheng M, Zhao P, Wang Y, et al. (2022) Preparation, characterization and application of poly (lactic acid)/corn starch/eucalyptus leaf essential oil microencapsulated active bilayer degradable film. *Int J Biol Macromol* 195: 264-273.