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Assessing the Antimicrobial, Antioxidant and Anti-inflammatory Potential of Ethanolic Extract of Irradiated *Rosa canina* L. Fruits

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SINCE, gamma-radiation (γ-ray) is an efficient refinement approach to keep and potentiate the plant's bioactive ingredients and biological activity, the influence of γ-ray (1, 3 and 5 kGy) on *Rosa canina L.* was investigated. Findings revealed that the ethanolic extract of irradiated and non-irradiated *R. canina* exert antimicrobial activity against all the pathogens tested to varying degrees. For all of the strains examined, the minimum inhibitory concentrations (MICs) varied from 16 to 64 µg ml^{-1}. Interestingly, the strongest inhibitory activity was recorded when *R. canina* was subjected to 1.0 kGy. Furthermore, irradiation enhanced the total phenolic content, showinga maximum increase for *R. canina* irradiated at 1.0 kGy. The antioxidant activity was also increased when compared to non-irradiated *R. canina*. Accordingly, the anti-inflammatory efficacy of the ethanolic extract of *R. canina* subjected to 1.0 kGy (ERC) was determined on D-Galactosamine (D-GalN/γ-ray) in the rat model of acute hepatitis (AH). Theresults showed that ERC significantly improved liver function enzymes and healed the histopathological distortion of the hepatic architecture. Hepatic oxidative stress was repressed, as evidenced by the decrease in MDA, with a concomitant increase in the antioxidant status including SOD, CAT, GPx, GST activities and GSH levels. Additionally, ERC significantly reduced the inflammatory response of iNOS, NO, IL-1β, and TNF-α; promoted Nrf2 translocation to the nucleus and triggered the production of cytoprotective expressing HO-1 and NQO-1. It is concluded that γ-ray potentiates the hepato-protective effect of *R. canina* to combat acute hepatitis-associated inflammation via antioxidant effects, inducesdetoxification enzymes, and activates the Nrf2/HO-1 pathway.

Keywords: Acute hepatitis,Anti-inflammatory, Antimicrobial, Nrf2/HO, *Rosa canina* L. fruit, γ- radiation.

Introduction

Infectious diseases represent an important cause of morbidity and mortality among the populations, particularly in developing countries (Joo et al., 2010). Owing to the constant emergence of microorganisms resistant to conventional antimicrobials, pharmaceutical companies have been motivated to develop new antimicrobial drugs in recent years (Fair & Tor, 2014). The application of bioactive compounds of natural origin has a long tradition. They have been widely used in traditional medicine worldwide as well as in the treatment and prevention of certain disorders (Rovná et al., 2020).

Acute hepatic failure is characterized clinically by severe hepatocyte apoptotic and extremely significant mortality rates. Multiple factors contribute to hepatic failure, including extremehabit of alcohol, virus-related diseases; transfer liver failure, the detrimental consequences of chemical medicines and xenobiotic metabolism (Trautwein & Koch, 2013). Indeed, an experimental approach of organ failure generated in rodents by D-galactosamine

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(D-GalN) /Lipopolysaccharide (LPS) and D-GalN/ γ -rays was found to replicate acute hepatic failure in medical settings (Takamatsu et al., 2018; Wanless, 2020). D-GalN combined with LPS or ionizing radiation causes liver damage in these experimental models via promoting reactive oxygen species (ROS), notably in Kupffer cells (Ibrahim et al., 2020; Nuszkiewicz et al., 2020). ROS over-generation is not only harmful to cells, it also leads to pathologicalprocesses indluding oxidation stress (Cichoż-Lach & Michalak, 2014). Medicinal plants emerge as alternative products, which are used not only in traditional medicine, but also in a number of food and pharmaceutical products due to their nutritional value and bioactivity. Many plant species of the Rosaceae family are considered to be of a high importance because of their use in various applications. *Rosa* is a genus of almost 200 species found in temperate and subtropical settings throughout Europe, Asia, North America, and the Middle East (Ercisli et al., 2007; Polumackanycz, et al., 2020). Fruits of *Rosa* spp. are rich in bioactive compounds with antioxidant activity, especially in flavonoids, tannins, carotenoids, mineral compounds, phenolic acids, fatty and organic acids. The species *Rosa canina* L., also called dog rose, is a plant that is a rich source of biologically active compounds with chemoprevention, antioxidant, antimutagenic, and anticarcinogenic activities (Yoruk et al., 2008; Karikas, 2010).

The current study has two goals: the first is to evaluate the use of gamma radiation atdifferent doses to improve the antimicrobial and antioxidant potentiality of *R. canina* fruit, and the second is to highlight the protective effects of *R. canina L.* against acute hepatitis induced by D-galactosamine and ionizing radiation.

Materials and Methods

Materials

Sigma–Aldrich® provided D-galactosamine andall reagents utilized in this investigation (St. Louis, Missouri, USA).*Rosa canina L.* fruits were obtained from a local store.

Irradiation processes

The fruits were washed, dried in a forced air oven at 40°C (Koca et al., 2009) and ground then, packaged in polyethylene pouches (each portion 500 g) to perform the irradiation. For the irradiation treatments, the powder was exposed to γ- irradiation at dose levels of 1, 3 and 5 (kilo Gray) kGy using ^{60}Co from the Gamma Chamber 4000 unit at the National Center for Radiation Research and Technology (NCCRT, Atomic Energy Authority, Egypt). The dose rate at the time of the experimentation was 2.3 kGy/h.

Preparation of the ethanolic extract

500 g of each non-irradiated and irradiated *R. canina* (1, 3 and 5 kGy) were combined in a Soxhlet device with 1 Liter of 70% ethanol and left for 48 h at 25°C. The supernatant fluid from the solution was then separated and filtered before being placed in a sterile container. The extract was filtered under reduced pressure in a rotary evaporator model RE52A, China (İlbay et al., 2013). The extract was then weighed and dissolved in double-distilled water with the use of a cyclomixer, to produce crude extracts, which were then refined for *in vitro* and *in vivo* bioactivity investigation.

Assessment of antimicrobial activity of ethanolic extract from irradiated and non-irradiated R. canina

Pathogenic strains

Eight bacterial strains were used in the present study to test the antimicrobial activity of ethanolic extract of *R. canina* (irradiated and non- irradiated). Four of them were Gram-negative (*Escherichia coli*, *Kelbseilla pneumoniae, Pseudomonas aeruginosa* and *Salmonellatyphimurium*) and the other four were Gram positive (*Staphylococcus aureus, Listeria monocytogensis, Bacillus cereus* and *Enterococcus faecalis*). For the antifungal activity; two*Candida spp. Candida albicans* and *Candida tropiclais* were applied. All the tested strains were previously isolated and characterized at the Microbiology Department, NCRRT. Cultures were grown on Nutrient agar (NA) plates for bacterial strains and Sabouraud-Dextrose agar (SDA) for Candida spp. and maintained in the agar slants at 4°C.

Antimicrobial assay

The bacterial strains were grown overnight at 37°C in Nutrient agar, while Candida spp. were grown in Sabouraud agar. The inoculum for the assays was prepared by inoculating three to five colonies from an agar plate culture into 10 mL of nutrient broth and then incubated at 37°C for 24 h. After growing, the microbial suspension

was standardized with a sterile saline solution to a turbidity equivalent to 0.5 McFarland scale (10^7 CFUmL^{-1} for bacteria and 10^5 CFUmL^{-1} for *Candida spp.*). The antimicrobial activity of the extracts was assayed using the agar well-diffusion method on Mueller Hinton Agar medium (MHA). For this, 0.1 mL of microbial strain was spread on the surface of MHA evenly, wells of 6 mm in diameter were punched into the agar and filled with 50 µL of ethanolic extract of the irradiated (at 1.0, 3.0 & 5.0 kGy) and non- irradiated *R. canina*. The plates were first kept at 4°C for at least 2 h to allow the diffusion of any antimicrobial metabolites and then incubated at 37°C for 24 h. Amoxicillin/Clavulanic Acid (AMC) and Nystatin (NS); were examined as standard antibiotics (positive control). All experiments were carried out in triplicate. The antimicrobial activity was determined by measuring the zones of inhibition (ZOI) (Neggaz et al., 2015).

Determination of Minimum Inhibitory Concentrations (MICs)

The MICs values were determined by the microtiter broth method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2008) in sterile flat-bottom 96-well polystyrene plates. Serial dilution technique**s** were used to determine the MICs of ethanolic extract of the irradiated *R. canina* (1.0 kGy) which ranged from 5 to 256 µgmL^{-1}. A final concentration of the tested organisms ($3x10^6$ CFUmL^{-1}) was added to each dilution. The plates were incubated at 32°C for 18 h. MIC was defined as the lowest concentration of antimicrobial agent that inhibited the microbial growth, as indicated by the absence of turbidity. Each test included two growth controls consisting of the medium with microbial suspension. The experiment was carried out in triplicate and at least on three different occasions.

Biofilm formation assay by tissue culture plate method (TCP)

Biofilm formation was determined using a spectrophotometric method described by Christensen et al. (1985). Briefly, stationery 18 h. culture of the tested microbial strains in 5 mL of Tryptone soya broth (TSB) were washed, diluted using fresh medium and standardized to contain about 10^6 CFUml^{-1}. Aliquots of 0.2 mL of the diluted cultures were added to the wells of the sterile flat bottom polystyrene tissue culture plates. Following 48 h. of incubation at 37°C, the contents of tissue culture plates were gently aspirated with a micropipette. The plates were then washed with sterile buffer. Adherent organisms were fixed by incubating them for 1 h. at 60°C (Baldassarri et al., 1993) and then staining them with crystal violet (0.1%) for 5 min. After washing using water to remove the excess stain, the plates were dried for 30 min at 37°C. Then, after drying the optical densities (OD595) of strains, adherent biofilms were read with Microplate Reader-SunoStick SPR-960B at 595 nm. Adherence measurements were performed in quadruplicate and repeated at least three times, the values were then averaged. Biofilm intensity was classified as weak, moderate and strong, the range 0.139- 0.276 was considered low biofilm-forming strains, 0.277- 0.414 was considered medium biofilm-forming strains and >0.415 was considered high biofilm.

Anti-biofilm activity of ethanolic extract of irradiated R. canina

The anti-biofilm activity of ethanolic extract for the irradiated *R. canina* (1.0 kGy) was evaluated through testing its ability to prevent the bacterial adherence by spectrophotometric method as follows: In each well of tissue culture plate 150 µL of 18 h. broth culture of the applied strains and 50 µl of the tested extract were mixed at the same time. In the case of control wells, 50 µL of sterile buffer was used and the procedure was conducted as described above.

Estimation of total phenolics content

The total phenolics content of the ethanolic extract of the irradiated R. *canina* (1, 3 and 5 kGy) and non-irradiated were quantitatively determined with the Folin–Ciocalteu reagent according to the method of Waterman (1994). In this method, 0.1 mL of extract diluted tenfold with deionized water (to obtain an absorbance in the range of the prepared calibration curve) was transferred to a test tube and mixed with 0.25 mL of Folin–Ciocalteu reagent (previously diluted tenfold with deionized water). The mixture was left at room temperature for 2 min. Then 1.25 mL of 20% sodium carbonate solution was added to the mixture, mixed gently, shaken thoroughly, and 0.5 mL of water was added. The mixture was left to stand for 40 min and the blue color developed was measured at 725 nm using a UV–Vis spectrophotometer. A calibration curve of gallic acid was used as a standard. The total phenolic content was expressed as milligram of gallic acid equivalents per gram of extract.

Antioxidant activity (DPPH Radical Scavenging Assay)

The antioxidant efficiency of the ethanolic extract ofthe irradiated *R. canina* (1, 3 and 5 kGy) and non-irradiated was estimated by measuring their capacity to scavenge the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical as described by Singh & Dhiman (2015). A definite amount of each sample was incubated with a methanolic solution of DPPH (2 mM) for 45 min in dark at room temperature then after the absorbance was measured at 517 nm using a UV-Visible spectrophotometer (UV-Analytic Jena AG specord 210 plus). The control was conducted in the same manner, except that distilled water was used instead of the sample. The DPPH radical scavenging activity was calculated as follows: DPPH scavenging %= $(A_B-A_H)/A_B \times 100$

Where A_B is the absorption of the blank (methanolic DPPH solution) and A_H is the absorption of the sample (sample in methanolic DPPH solution). Lower absorbance of the reaction mixture indicated a higher DPPH radical-scavenging activity. The test was carried out in triplicate.

Experimental animals

In the present study, 5 weeks-old Wister male rats (150± 20 g) were obtained from the Egyptian National Authority for Drug Research and Control, Cairo, Egypt. The animals were maintained in a controlled environment under standard conditions of temperature (25 ± 2°C), humidity and light (12 h/24 h). The animals received a standard diet and water ad libitum. Experimental methods have been conducted according to the National Research Center Ethics Committee standards and guidelines issued by the U.S. National Health Institutes (NIH publication No. 85–23, 2011) and the usage of experimental animals licensed by the National Centerfor Radiation Research and Technology (NCRRT) animal care committee, Cairo, Egypt.

Acute hepatitis induction and treatment with ethanolic extract of Rosa Canina L.

The experimental animals were divided into four main groups, with six rats in each group (n= 6). 1-Control group, rats received orally 1.0 mL saline/day for 4 weeks. 2-Acute hepatitis (AH) group, rats were injected i.p with a single dosage of D-GalN 300 mg in 1.0 mL saline/kg b.wt (Nagakawa et al., 1993). Following the dosage of D-GalN, rats were exposed to a single dose (2 Gy) of γ-rays (Ghorbani et al., 2020). 3-*R. canina* (ERC) group, rats were administered orally 500 mg ethanolic extract ofirradiated *Rosa canina L.* Fruitsin 1.0 mL saline/kg b. wt./day for 30 days. 4- *R. canina*+AH group, rats were administered orally with *R. canina* as in group3one month before induction of acute hepatitis. After two days from D-GalN/ γ-ray -induced acute hepatitis, rats fasted overnight before the cervical dislocation sacrifice period. Samples of the blood were collected in sterile heparinized and non-heparinized tubes via the heart puncture. The entire animal liver was separated immediately, rinsed with ice-cold saline, and split into two sections. For the histological study, the first sections have been placed in a 10% formalin solution and coated in paraffin, while the second section was frozen immediately in liquid nitrogen and stored at -80°C for analysis.

Irradiation processes in vivo

Rats' whole-body gamma irradiation was performed at the National Center for Radiation Research and Technology (NCRRT) of the Egyptian Atomic Energy Authority, Cairo, Egypt using an indoor shielded Canadian gamma cell-40 (Cesium-^{137}) at a dose rate of 0.67 Gy min^{-1}.

Biochemical measurements

The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were evaluated using rat ELISA kits (Bio Diagnostic Company, Egypt). Tissues' glutathione-S-transferase (GST) enzyme activity was measured according to the method described by Habig et al. (1974). The glutathione peroxidase (GSPx) in liver homogenate was estimated using the Gross et al. (1967) technique, which is based on measuring the quantity of residual glutathione (GSH) that remains after a predetermined duration of exposure to enzyme activity. Hepatic malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase (CAT) activities, and GSH content were measured as previously described using commercially available colorimetric assay kits (Bio-diagnostic Co., Egypt).Nitric oxide (NO) was determined in serum and was measured indirectly as nitrite using Griess reagent according to Moshage et al. (1995). Using rat ELISA kits (CUSABIO Technology Biotech Co., China), the levels of tumor necrosis factor (TNF-α, cat. no. CSB-E11987r) and interleukin 1beta (IL-1β, cat. no.

CSB-E08055r) in liver tissues were measured. Further more, commercial kits were used to evaluate Nuclear Factor-E2-related factor 2 (Nrf-2, MBS752046) in liver tissue homogenates using sandwich ELISA (MyBioSource, Inc., USA). The rat ELISA kits provided by CUSABIO, Wuhan, China, were used to evaluate the nuclear factor kappa B (NF-B p-65, Code CSB-E08788r) and heme oxygenase 1 (HO-1, Code CSB-E08267r) levels, respectively. The absorbance at 450.0 nm was determined using a microplate reader (model 680Bio-Rad, USA).

Molecular investigation

The quinone oxidoreductase 1 (NQO-1) and inducible nitric oxide synthase (iNOS) gene expression level were evaluated, using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) using (Life Technologies, Thermo Fisher Scientific Inc., USA) instrument according to Livak & Schmittgen (2001). The sequences of PCR primer pair were as the Table:

Table primer sequences used for RT-PCR

Rat primer	Sequence
NQO-1	Forward:5′-AGGCTGGTTTGAGCGAGT-3′ Reverse: 5′-ATTGAATTCGGGCGTCTGCTG-3
iNOS	Forward:5′-CAACAACACAGGATGACC CTAA-3′ Reverse: 5′-GGTAGGTTCCTGTTGT TTCTAT-3′
β-actin	Forward:5′-TGTTTGAGACCTTCAACACC-3′ Reverse:5′-CGCTCATTGCCGATAGTGAT-3′

Histopathological study

Liver tissue specimens were fixed in 10% formol saline, then trimmed off, washed and dehydrated in ascending grades of alcohol. The dehydrated specimens were then cleared in xylene, embedded in paraffin blocks and sectioned at 4-6 µm thick. The obtained tissue sections were deparaffinized using xylol and stained using hematoxylin and eosin (H&E) for histopathological examination through the electric light microscope according to Bancroft & Turner (2013). The histopathological findings were score of necrobiotic changes of hepatocytes and degree of periportal lymphocyte infiltration according to Ramos et al. (2015).

Statistical analysis

The hepatic lesions were scored according to the following criteria:

Score	Description
0	Minimal or no evidence of injury
I	Hydropic degeneration
II	Micro-vesicular steatosis
III	Necrosis (hypo-stained or absent nucleus, intense cytoplasmic eosinophilia, and destruction or loss of the architecture of the hepatocyte cord)
IV	Apoptosis.

Degree of periportal and peri-septal lymphocyte infiltration:

Degree	Septal and portal inflammatory infiltrate
0	Absence or rare portal lymphocytes
1	Discreet increase in the number of portal lymphocytes
2	Moderate increase in the number of portal lymphocytes
3	Marked increase in the number of portal lymphocytes
4	Strongly marked increase in the number of portal lymphocytes

The SPSS (version 20) was used in data analysis. Data were analyzed using one-way analysis of variance (ANOVA) followed by a post hoc test (LSD) for multiple comparisons. P values <0.05 were considered statistically significant.

Results and Discussion

Several scientists have been seeking for a technology that would not develop resistance to novel and efficient antimicrobial agents, in order to prevent bacteria from developing single or multiple antibiotic resistances and to provide affordable health care. Plant extracts have been used for many thousands of years in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens (Kumar et al., 2012).

Antimicrobial activity of R. canina extracts

The antimicrobial activity of ethanolic extracts of irradiated (1,3, and 5 kGy) and non- irradiated *R. canina* against ten pathogenic strains using well diffusion assay are presented in Table 1. The results revealed that all the ethanolic extracts showed antimicrobial activity against both Gram- positive, Gram -negative bacteria and *Candida* spp. The extracts have different degrees of inhibitory activity, depending on the strain and radiation dose. In general Gram- positive bacteria under study were the most susceptible to all extracts, the diameter of inhibition zones ranged from (16.3-22.1 mm). While the extracts had moderate antimicrobial activity against Gram negative bacteria *P. aeruginosa, E. coli and K. pneumoniae*, the extracts showed less activity towards *S. typhi* especially with high doses. It is interesting to note that the extract from irradiated *R. Canina*at 1.0 kGy showed the significant strongest inhibitory activity ($P<0.05$) against all the tested pathogens with zone of inhibition ranging from 15.3-26.1 mm, while, other doses exhibited a remarkable decrease in inhibition zone ranged from 11.3-19.2 mm and 10.8- 13.9 mm with 3.0 and 5.0 kGy, respectively compared to non- irradiated *R. canina* where the inhibition zone ranged from 11.6-22.1 mm.

The antimicrobial activities of many plant extracts have been previously reviewed and classified as strong, medium or weak (Kumar et al., 2012). The present study reveals the antimicrobial potential of ethanolic extracts from *R. canina* (irradiated and non-irradiated). Almost all extracts of the plant showed antibacterial potential and produced zone(s) of inhibition. Extracts from *R. canina* fruitdemon strated antibacterial activity thus; it should be further studied to determine the active component(s). Furthermore, Gram- negative bacteria were found to have less susceptibility as compared to Gram-positive bacteria species and Candida species. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms.

The antimicrobial activity of *R. canina* leaf extract was evidenced against *P. aeruginosa* and *S. typhimurium* (Živkovi et al., 2015). Rose fruits deserve special attention as a potential source of natural antioxidants and other bioactive substances, they have been discovered to be rich in polyphenols, polysaccharides, essential oil, polyunsaturated fatty acids (in seeds), vitamin A, B1, B2, B6, D, E, and K, and mineral nutrients (Cendrowski et al., 2020). The authors also stated thatthe polyphenols present in plant extracts, such as flavonoids or tannins, due to the presence of -OH groups, tend to incorporate into microorganism's membrane and cell wall, which, in turn, leads to a change in their fluidity and permeability. Polyphenolic compounds can also inhibit the synthesis of DNA and RNA, polysaccharides, enzymes, and proteins. This causes a violation of the enzyme system as well as a weakening of biochemical stability and a weakening of the membrane potential, which, in consequence, contributes to the death of the microbial cell.

TABLE 1. Antimicrobial activity of ethanolic extract of irradiated *R. canina* against different microbial strains

Microbial strains	Mean of inhibition zone (mm)					
	Control	1.0 kGy	3.0 kGy	5.0 kGy	AMC*	NS**
Gram –ve						
P. aeruginosa	13.2 ±0.15	16.2±0.11	13.4 ±0.13	10.9 ±0.22	17.2±0.15	NT
E. coli	15.2±0.29	17.2±0.15	14.1±0.13	12.2 ±0.15	16.5 ±0.13	NT
K. pneumoniae	14.3±0.05	16.2±0.09	15.5±0.18	11.2±0.17	18.2 ± 0.08	NT
S. typhi	11.6±0.19	15.3 ±0.15	11.3 ±0.14	10.8 ±0.01	15.0 ±0.00	NT
Gram +ve						
S. aureus	22.1±0.08	26.1±0.09	19.2±0.11	13.9 ±0.25	21.2±0.12	NT
B. cereus	17.2±0.15	23.4±0.18	16.8±0.15	12.1±0.05	18.5±0.15	NT
Ent. faecalis	19.2 ±0.06	25.1±0.09	17.5 ±0.12	11.6±0.08	18.7± 0.03	NT
L.monocytogensis	16.3 ±0.15	19.2±0.11	15.6±0.04	12.6±0.22	17.2±0.05	NT
Unicellular-fungi						
C. albicans	18.2 ± 0.23	23.4 ±0.12	16.3 ±0.15	12.4 ±0.09	NT	Negative
C. tropicalis	17.6±0.13	20.5±0.05	15.4 ±0.01	11.3 ±0.13	NT	Negative

Values are mean ± SE (n = 3). NT: Not tested; Negative:no ZOI had been measured.

AMC= Amoxicillin/Clavulanic Acid; 20/10 µg/mL (Antibacterial standard). NS=Nystatin (Antifungal standard).

Previous studies documented that the methanolic extracts of fruits of various Rosa spp. plants showed varying antibacterial activities against different microorganisms (Rovná et al., 2020). The results of the MICs of ethanolic extract of irradiated *R. canina* at 1.0 kGy (Table 2) showed that the lowest MIC of the extract was against *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *Ent. faecalis* (16 µgmL^{-1}) followed by *E.coli* and *B.cereus* of (32 µgml^{-1}). While, *S. typhi* L. *monocytogensis* and Candida spp. were (64 µgmL^{-1}). Oyedemi et al. (2016) reported that the MIC of methanolic extract of *R. canina* was between 256 and 512 mg/mL against MRSA and MDR bacterial strains. In another study with *R. canina*-mediated biogenic silver nanoparticles, MICs ranged between 16 µg/mL and 256 µg/mL for *Bacillus cereus, Enterococcus hirae, Staphylococcus aureus, Escherichia coli, Legionella pneumophila, Candida albicans,* and *P. aeruginosa* (Gulbagca et al., 2019).

TABLE 2. Minimum inhibitory concentrations of ethanolic extract of irradiated*R. canina* at 1.0 kGy

Microorganisms	MIC µg/mL^{-1}
Gram –ve	
P. aeruginosa	16
E. coli	32
K. pneumoniae	16
S. typhi	64
Gram +ve	
S. aureus	16
B. cereus	32
Ent. faecalis	16
L.monocytogensis	64
Unicellular-fungi	
C. albicans	64
C. tropicalis	64

Biofilm formation assay

Biofilm-linked persistent infections are not easy to treat due to resident multidrug-resistant microbes. Low efficiency of various treatments and in vivo toxicity of available antibiotics drive the researchers toward the discovery of many effective natural anti-biofilm agents. Natural extracts and natural product-based antibiofilm agents are more efficient than the chemically synthesized counterparts with lesser side effects (Mishra et al., 2020).

Mathur et al. (2006) reported that the TCP method is considered an accurate method for screening and determination of biofilm production. Therefore, in the current study, all the strains were subjected to quantitative assessment of biofilm formation by spectrophotometric method. Table 3 shows that all the tested strains are considered strong biofilm-forming strains under the conditions utilized in the present experiment, except two species of *Candida* which were considered moderate biofilm-forming strains. Moreover, the data demonstrated that ethanolic extract of the irradiated *R. canina* treatment reduced the biofilm formation. All the tested strains displayed a highly significant decrease in biofilm formation ($P< 0.05$) with the treatment especially with *K. pneumoniae* and *Ent. faecalis* where inhibition percentage reached 83.0 and 75.8%, respectively.

Yong et al. (2019) reported that there are broadly five classes of natural compounds that have high anti-biofilm properties. Those are phenolics, essential oils, terpenoids, lectins, alkaloids, polypeptides, and polyacetylenes, these entire compounds act on biofilm by six main mechanisms includingsubstrate deprivation, membrane disruption, and binding to adhesin complex and cell wall; bind to proteins; interact with eukaryotic DNA; and block viral fusion (Cowan 1999; Lu et al., 2019).

Determination of total phenolic contents

Phenolic compounds are the most bioactive factors in plants. They are known to act as antioxidants not only because of their ability to donate hydrogen or electrons, but also because they are stable radical intermediates (Nepote et al., 2000). The data in Table 4 shows the amount of the total phenolic compounds (mg/100g gallic acid) in ethanolic extracts of irradiated and non-irradiated *R. canina* at 1, 3 and 5 kGy.

The results showed that the control sample possesses a marked total phenolic compound 20.07,while, subjecting samples of *R. canina* to gamma irradiation at 1 and 3 kGy increased the amounts of phenolic compounds by 33.79 and 22.81 respectively; compared with the control sample. Whereas, the total phenolic content was reduced with 5 kGy to reach 11.29,the data exhibited significant differences between the control and irradiated samples at dose of 1.0 kGy. However, there were insignificant differences in the irradiated sample at dose levels 3.0 kGy.

TABLE 3. Inhibition percentage of biofilm formation after treatment with ethanolic extract of irradiated *R. canina* at 1.0 kGy

Tested strains	Biofilm formation ability (O.D)*	Biofilm formation after treatment with (ERC*)	Inhibition (%)
Gram –ve			
P. aeruginosa	1.455 ±0.003	0.440 ±0.001	69.76
E. coli	1.005 ±0.004	0.390 ±0.006	61.19
K. pneumoniae	1.680 ±0.003	0.290 ±0.005	83.00
S. typhi	0.880 ± 0.020	0.416 ±0.004	52.73
Gram +ve			
S. aureus	0.760 ±0.002	0.460 ±0.002	39.47
B. cereus	0.490 ±0.025	0.123 ±0.001	73.26
Ent. **faecalis**	0.960 ±0.150	0.232 ±0.012	75.83
L.monocytogensis	0.554 ±0.001	0.202 ±0.005	63.53
Unicellular-fungi			
C. albicans	0.400 ±0.030	0.223 ±0.004	51.52
C. tropicalis	0.330 ±0.008	0.138 ±0.001	58.20

*Values are mean of three replicates ±SE. *(ERC): ethanolic extract of irradiated *R. canina*.

*Biofilm intensity was classified as weak, moderate and strong, (0.139- 0.276) were considered low biofilm-forming strains (0.277-0.414) were considered medium biofilm-forming strains and (>0.415) were classified as high biofilm.

TABLE 4. Total phenolic compounds (mg/100 g as Gallic acid) of ethanolic extracts of irradiated and non-irradiated *R. canina*

Total phenolic compound (mg GAE/g)	Control	Gamma irradiation dose (kGy)		
		1.0	3.0	5.0
R. canina	20.07±0.015 ^{a}	33.79±0.001 ^{b}	22.81±0.02 ^{a}	11.29±0.005 ^{c}

Values are mean of three replicates ± SE. Mean values followed by different superscript (within the same row) is significantly differentat the 5% level.

Comparing the results obtained in the present study to those found in the literature, Wenzig et al. (2008) found lower TPC in water extracts of commercial samples of rose hip with fruits and without fruits, 3.7 and 2.7 µg GAE/g, respectively, and higher TPC in methanolic extracts, 133 and 82.2 µg GAE/g, respectively. Regarding the dog rose leaves, Querghemmi et al. (2016) found TPC in methanolic extracts on low level, 255 µg GAE/mg dry extract.

Radical scavenging activity (DPPH assay) of ethanolic extracts from irradiated and non-irradiated R. canina

The antioxidant activity of the phenolic compounds may result from the neutralization of free radicals initiating oxidation processes or from the termination of radical chain reactions. Furthurmore, the antioxidant activity of phenolics is due to their high tendency to chelate metals.

Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups (Heim et al., 2002).

The results in Table 5 indicated that there is a marked increase in the DPPH scavenging activity for all the evaluated extracts from irradiated *R. canina* at 1.0, 3.0 and 5.0 kGy compared to that of the non-irradiated sample. These findings come in good agreement with similar studies reported by Adamo et al. (2004) and Zantar et al. (2015) in which they attributed the increase in the antioxidant activity after irradiation to the degradation of molecules to other phenolic molecules or because of the changes in the conformation of molecules that contribute in antioxidant activity.

Radiation treatments have been shown to either increase or decrease the antioxidant content of fresh plant products, which is dependent on the dose delivered, exposure time and the raw material used. The enhanced antioxidant capacity of a plant after irradiation is mainly attributed either to the increased enzyme activity (e.g., phenylalanine ammonialyase and peroxidise activity) or to the increased extractability from the tissues (Bhatt et al., 2007). Khattak (2012) reportedthat γ- irradiation of *Emblica officinalis*

increased the levels of certainphyto chemicals and the free radical scavenging activity was enhanced in all the radiation-treated samples up to the dose levels of 12 kGy.

Polumackanycz et al. (2020) stated that fruits of *Rosa canina* L. (dog rose) are rich source of phenolic compounds and antioxidant agents which positively affect the human health and offer great hope for the prevention of chronic human diseases.

This study indicated that the tested extract may possess antimicrobial activity and can be exploited as an ideal treatment for future human disease management programs eliminating microbial spread. In brief, the current study suggests that γ-irradiation is effective to improve the quality of plant material. Aside from the impressive *in vitro* results mentioned above, several biochemical markers, including histological study of the liver, were investigated to identify the severity of hepatitis and the influence of irradiated *R. canina:*

- Control (A) and ethanolic extract of irradiated *R canina* 1 kGy (B) showing normal architecture of hepatic lobules and radiating plates of polygonal cells with prominent round nuclei (arrow).

- Acute hepatitis showing numerous numbers of eosinophilic apoptotic bodies (arrow) and focal leukocytic infiltration (arrow head) (C) and portal lymphocytes infiltration and hyperplasia of bile duct epithelial lining (arrow) (D)

- Acute hepatitis treated with the ethanolic extract of irradiated *R canina* 1 kGy showing mild swelling and granularity of cytoplasm (arrow) with hyperplasia of Kupffer cells (E) and little number portal lymphocytic infiltration(arrow) (F) (H & RX200).

Figure 1 (A & B) showsliver tissue sections of the control (Fig. 1A) and ethanolic extract of irradiated *R canina* 1kGy (Fig.B) groups revealing normal architecture of hepatic lobules made up of radiating plates of polygonal cells with prominent round nuclei and eosinophilic cytoplasm (score 0). Sinusoids lined by fenestrated endothelial cells with fine arrangement of Kupffer cells. The portal area revealed normal histological structure of bile duct, portal vein and hepatic artery (degree 0). On the other side, Fig. 1 (C & D) shows the hepatitis induced group revealing disorganization of hepatic cords and necrobiotic changes of hepatocytes characterized by numerous numbers of eosinophilic apoptotic bodies and focal leukocytic aggregation mainly lymphocytes and macrophages score IV. Strongly marked increase in the number of portal lymphocytes infiltration and hyperplasia of bile duct epithelial lining were seen (degree 4). Figure 1 (E & F) shows the hepatitis induced group treated with the ethanolic extract of the irradiated *R canina* showing a marked improvement of hepatic parenchyma characterized by organization of hepatic cords. The hepatocytes showed mild swelling and granularity of its cytoplasm with hyperplasia of Kupffer cells (score I). Reduction portal lymphocytic infiltration was noticed (degree 1).

The pseudo-fruits of Rosa genus plants in the Rosaceae family are known as rose hips. The Rosa genus contains around 100 species which are widely spread across Europe, the Middle East, Asia and North America (Mármol et al., 2017), and was instrumental in attenuating liver injury in rats induced by a D-GalN/ γ-ray exposure. In the present study, acute hepatitis resulted in a significant elevation in serum marker enzymes, ALT, AST, and ALP, compared to the control group, indicating liver injury. Besides, severe liver lesions were also noticeable, hepatic cord disruption and necrobiotic alterations in hepatocytes as seen with many eosinophilic apoptotic bodies and localized leukocytic aggregation, mostly lymphocytes and macrophages (Fig. 1) in histopathological findings. This is consistent with earlier findings that the increased enzyme levels reveal inflammatory and necrotic conditions in the hepatic cells (Ibrahim et al., 2020). All of the above-mentioned pathological changes were significantly abrogated with irradiated *R. canina* treatment, suggesting that pretreatments with irradiated *R. canina* were able to alleviate AH injury in rats. This could be because *R. canina* treatment stimulates the repair of parenchymal liver cells, hence preserving cell membrane integrity and reducing enzyme leakage. *R. canina* considerably enhanced serum antioxidant capability and vitamin C concentration. The extract can reduce reactive oxygen species in cellular and cellular systems (Sadigh-Eteghad et al., 2011). Antioxidant properties are attributed to vitamin C and polyphenolics compounds; alsoit isan abundant source of carotenoids.

TABLE 5. Scavenging activity (%) on DPPH radical of ethanolic extracts from the irradiated and non-irradiated *R. canina*

Concentration of samples (µg/mL)	TBHQ	Doses of gamma radiation (kGy)			
		0.0	1.0	3.0	5.0
25	70.12	27.16±0.005	37.20± 0.002	32.01± 0.005	29.03± 0.001
50	84.03	35.05±0.020	39.75± 0.013	39.15± 0.021	37.18± 0.015
100	95.82	41.95±0.011	53.19± 0.003	46.26± 0.013	43.56± 0.009

TBHQ= Tert-Butylhydroquinone

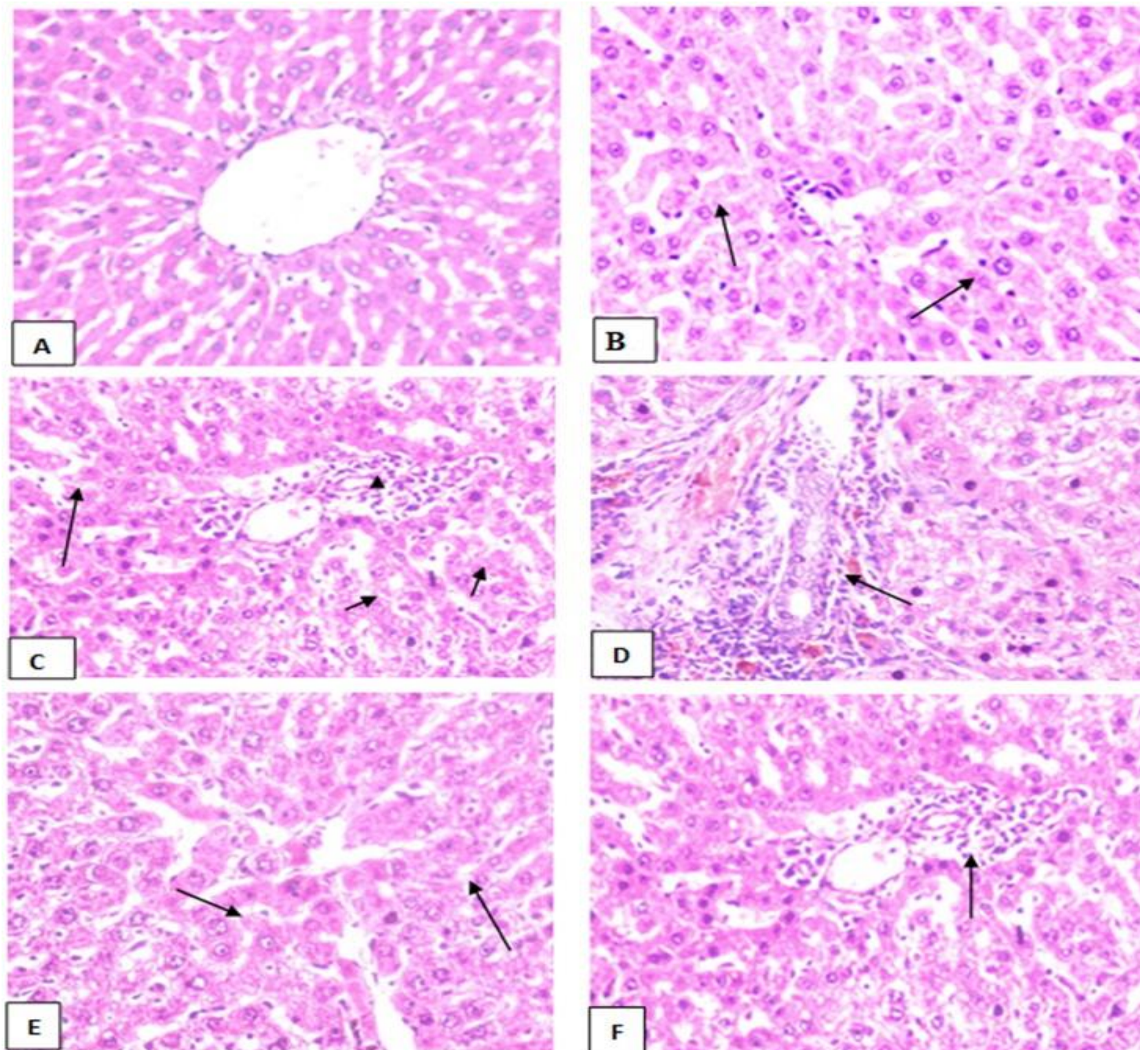

Fig. 1. Photomicrograph of hepatic tissue sections

MDA is a lipid peroxidation indicator and the end result of polyunsaturated fatty acid oxidative breakdown. Table 6 shows that the D-GalN/ γ-ray (AH) group considerably (P<0.01) raised hepatic MDA levels. In comparison to the AH group, ERC treatment significantly reduced levels of MDA in the hepatic tissues. Acute hepatitis was a popular animal model for studying potential hepatoprotective activity. As previously stated, the liver toxicity of D-GalN/ γ-ray is mediated through the production of ROS and a decrease in the hepatic pool of uracil nucleotides, a loss of intracellular calcium homeostasis, and a change in the membrane phospholipid composition are among the hepatotoxic effects of D-GalN. Hepatic cell necrosis is also caused by the activation

of local macrophages and the inflammatory process (Ibrahim et al., 2020). In addition, γ-radiation exposure causes immune response abnormalities and prolonged inflammation through deregulating inflammatory cytokines as well as stimulating pro-inflammatory pathways that alter tumor necrosis factor (TNF-α) and interleukin levels, resulting in degenerative processes (Moustafa et al., 2021). The fruit extract of *R. canina*, given at a dosage of 500 mg/kg, significantly reduced the increase in the hepatic MDA levels in the current investigation. It is worth noting that the fruits of *R. canina* are high in phenolic compounds, which have a wide range of biological and pharmacological properties, including anti-inflammatory and antibacterial properties (Winther et al., 2016).

The obtained results revealed that, in the AH group, there was a great decrease in the levels of GSH and SOD in the hepatic tissues when compared to the control group ($P<0.01$). In comparison to the AH group, *R. canina* treatment considerably ($P<0.05$) improved GSH levels. ERC administration at 500 mg/kg also enhanced SOD levels considerably ($P<0.05$) as compared to the AH group (Table 7). The activities of CAT, GPx, and GST in the liver tissues of the AH group were significantly ($P<0.01$) decreased when compared to the control group. In comparison to the AH group, ERC therapy significantly increased CAT and GPx activity ($P<0.01$). In comparison to the AH group, ERC considerably ($P<0.01$) boosted GST activity (Table 7). The hepatic antioxidant defence system disruption is characterized by increased MDA and/or altered enzymatic and non-enzymatic antioxidants including SOD, CAT, GPx, GST, and GSH. The action of SOD is to scavenge the superoxide anion via conversion to hydrogen peroxide and oxygen, which is further detoxified to water by CAT or GPx. GST is involved in several biological functions, including, the detoxification of alkylation of lipids, proteins, and nucleic acids, as well as providing cellular resistance to lipid peroxidation by increasing the conjugation of cytotoxic electrophilic with GSH. As a result, the activity of these enzymes has been utilized to measure cellular oxidative stress (Gullner et al., 2018). Furthermore, the excess reactive oxygen species (ROS) produced by D-Gla/γ-ray would disrupt the equilibrium between ROS generation and the antioxidant defence system. In the present study, interestingly, ERCmarkedly restored the activities of those antioxidant enzymes and the content of GSH in the acute hepatitis liver induced by D-GalN/γ-ray exposed rats. It is suggested that *R. canina* could attenuate the oxidative stress at least partly by renewing the activities of the antioxidant enzymes and increasing the content of GSH in the liver. *R. canina* has also been shown to have antioxidant properties in various tests (Fecka, 2009; Montazeri et al., 2011; Roman et al., 2013). Furthermore, the radical scavenging abilities of *R. canina*extracts were shown to be favorably linked with the phenolic content (Sadeghi et al., 2016). As a result, it is probable that the *R. Canina* fruit extract's hepatoprotective effect is mediated in part by the free radical scavenging activity.

The obtained results showed that AH- reduced hepatic Nrf2 protein level significantly ($P<0.001$) in rats. The suppressed Nrf2 signaling in D-Gla/γ-ray-intoxicated rats was confirmed by reduced NQO-1 and HO-1 gene expression ($P<0.001$). Rats treated with irradiated *R. Canina* (500 mg/kg) before AH showed a remarkable alleviation in the hepatic levels of Nrf2, NQO-1 mRNA, and HO-1 mRNA. A series of factors are believed to be involved in the pathogenesis of liver injury, including oxidative stress, inflammation, and immune reactions. Excessive ROS induced by AH exhibited nuclear factor kappa B (NF-κB) activation and subsequent nuclear translocation, which are responsible for modulation of liver injury by regulating inflammatory cytokine production, such as TNF-α, and the induction of inflammation associated enzymes, including iNOS (Karin et al., 2004). D-galactosamine / gamma radiation exposure generates excessive levels of nitric oxide (NO) through activation of iNOS, with an increase in the activity of cytokines, an index of hepatic inflammation, thus contributing to the damage in the hepatic tissues (Ghosh et al., 2012; Hassan et al., 2021). The present results indicated that *R. Canina* significantly normalized the NO content and iNOS gene expression levels (Table 8). Moreover, proinflammatory cytokines including IL-1β, IL-6, and TNF-alpha have been the focus of investigations into organ wounds since the uncontrolled and prolonged action of these proteins is injurious (Moustafa et al., 2020). Furthermore, Jeong et al. (2015) confirmed the anti-inflammatory effects of *Rosa canina* L. in a rat model of osteoarthritis by inhibiting the TNF,

IL-6, and cyclooxygenase-2, iNOS expression and reduced cytokine levels (IL-1, TNF-, and p-65). The phytosterol content, such as beta-sitosterol, inhibits the phosphorylation of NF-kB/TNF-stimulated human endothelial cells, which could explain the reductions in TNF-, IL-1, and NF-kB (p-65) levels after *R. Canina* administration. Phenolic compounds, such as gallic acid, re-establish the association of IκBα with NF-κB, hampering the capacity of NF-κB to bind DNA (Mármol et al., 2017).

By modulating the antioxidant response element which orchestrates adaptation to cellular redox disturbances, Nrf2 is a key regulator of intracellular redox homeostasis. The prospective use of Nrf2 as a targeted therapy to alleviate viral hepatitis, alcoholic and nonalcoholic disease, and fibrosis is extensively recognized (Xu et al., 2019). Numerous phytochemicals protect the liver by increasing the production of cytoprotective and/or antioxidant pathways via the Nrf2 signal transduction pathway, in addition to scavenging free radicals (Tu et al., 2019). Typically, the Kelth-like ECH-associated protein 1 (Keap1) anchors Nrf2 in the cytoplasm for proteasomal degradation. When activated by stimulants, Nrf2 dissociates from Keap1 and translocate into the nucleus, where it binds to the ARE of target genes encoding a variety of antioxidant enzymes and phase II detoxification enzymes. This causes the activity of Phase II detoxifying enzymes, including GST, NQO1, and HO-1 to protect hepatocytes against numerous toxins (Saha et al., 2020). In the current study, Nrf2 expression was notably increased by *R. canina* treatment compared with the AH group, suggesting the activation of the Nrf2 defence pathway. Moreover, *R. canina* treatment obviously enhanced expression of the signaling components, such as HO-1 and NQO1. Severe oxidative stress has been proposed as a cause of HO-1 overexpression, as this enzyme is known to be easily induced by such stressors. HO-1 is a rate-limiting enzyme in the catabolism of heme that belongs to a family of cytoprotective and detoxification genes that possess AREs in their regulatory regions. It is critical for keeping antioxidant equilibrium and limiting hepatotoxicity in acute liver disorders (Immenschuh et al., 2010). NQO1 is a phase II detoxifying enzyme that catalyses the two-electron reduction and detoxification of quinones as well as other redox-cycling endogenous and exogenous chemicals (Ross & Siegel, 2017). In the present study, *R. canina* treatment also reversed the expression of NQO1, which was down-regulated by AH. These results indicated that the protective effects of *R. canina* might be linked with the activation of the Nrf2 pathway and up-regulation of HO-1 and NQO1 (Table 9).

Conclusion

The current study indicated that the tested extract may possess antimicrobial activity and can be exploited as an ideal treatment for future human disease management programms to eliminate microbial spread. In brief, the study suggests that γ-irradiation is effective for the quality improvement of plant material. *R. canina* effectively ameliorates the AH-induced liver injury and inflammation in rats through alleviation of oxidative stress and induction of hepatic detoxification enzymes and efflux transporter expression, at least in part, via activation of the Nrf2/HO-1 pathway. Further studies are needed to determine the possible effect of *R. canina* on any other cell signaling pathways involved in the pathogenesis of AH.

TABLE 6. Effect of the ethanolic extract of irradiated *R canina* 1.0kGy (ERC) on AH-induced elevations in serum hepatic enzyme activities in rats

Groups	Control	ERC	AH	ERC+AH
ALT (U/L)	29.4 ± 0.51	31.5± 1.5[c]	50.8 ± 0.82 [a,b]	36.5[#]± 1.36 [a,b,c]
AST (U/L)	142.2±6.7	143± 0.63[c]	167.9±7.46 [a,b]	149.4[##]± 0.98 [a,b,c]
ALP (U/L)	5.1±0.24	4.8± 0.31[c]	10.5±0.16 [a,b]	7.02± 0.42 [a,b,c]

Data are means of 6 animals ± SE.
[a]P-value <0.05 vs. control group,[b]*P*-value <0.05 vs. ERC group, [c]P-value <0.05 vs. AH group.

TABLE 7. Effect of the ethanolic extract of irradiated *R canina* 1.0 kGy (ERC) on AH-induced alteration balance in antioxidant enzyme activities and lipid peroxidation in rats

Groups	Control	ERC	AH	ERC+AH
MDA (nmol/g tissue)	2.1 ± 0.11	1.9 ± 0.25 [c]	7.8 ± 0.32[a,b]	3.5± 0.09 [a,b,c]
GSH (µg/mg tissue)	28.3 ± 0.96	30.5± 1.54 [c]	17.1±0.76[a,b]	23.4± 1.18 [a,b,c]
GPX (U/mg tissue)	14.3±1.23	13.72± 0.58[c]	7.24± 0.80[a,b]	10.10± 0.44 [a,b,c]
GST (U/mg tissue)	316.7 ± 7.36	320.9 ± 21.5[c]	179.1 ± 6.9[a,b]	291.9 ± 10.7 [a,b,c]
SOD (U/mg tissue)	6.1±0.24	7.6± 0.41 [c]	4.0±0.16[a,b]	6.3± 0.15 [a,b,c]
CAT (U/mg tissue)	3.2± 0.10	3.70 ± 0.17 [c]	1.63± 0.08[a,b]	2.7 ± 0.11 [a,b,c]

Data are means of 6 animals ± SE.

[a]P-value <0.05 vs. control group, [b]P-value <0.05 vs. ERC group, [c]P-value <0.05 vs. AH group.

TABLE 8. Effect of the ethanolic extract of irradiated *R canina* 1.0 kGy (ERC) AH-induced inflammation responses in rats

Groups	Control	ERC	AH	ERC+AH
TNF-α (pg/mg tissue)	133.1 ± 4.11	129.9±9.25 [c]	218.0±5.32 [a,b]	163.5± 8.09 [a,b,c]
IL-1β (pg/mg tissue)	27.2±0.4	29.9± 1.54 [c]	137.1±0.76 [a,b]	63.4± 1.18 [a,b,c]
NO content (nmol/g tissue)	13.48± 1.25	14.23± 1.34 [c]	5.84± 0.23 [a,b]	9.21± 0.74 [a,b,c]
iNOSmRNA fold change	0.5± 1.25	0.4± 1.34 [c]	1.84± 0.23 [a,b]	0.6± 0.74 [b,c]
p-65 (pg/mg tissue)	38 ± 1.2	40.0 ± 1.0 [c]	104 ±3.5 [a,b]	50±2.0 [a,b,c]

Data are means of 6 animals ± SE.

[a]P-value <0.05 vs. control group, [b]P-value <0.05 vs. ERC group, [c]P-value <0.05 vs. AH group.

TABLE 9. Effect of the ethanolic extract of irradiated *R canina* 1.0 kGy (ERC) AH-changes hepatic Nrf2/HO-1 pathway

Groups	Control	ERC	AH	ERC+AH
Nrf2 (pg/mg tissue)	242.8±6.72	251.5±4.02 [c]	109.4±4.92 [a,b]	218.5±7.04 [a,b,c]
NQO-1mRNAFold change	1.0±0.01	1.0±0.02 [c]	0.35±0.05 [a,b]	1.1±0.03 [a,b,c]
HO-1mRNAFold change	1.02±0.01	1.05±0.02[c]	1.1±0.05 [a,b]	1.02±0.02 [a,b,c]

Data are means of 6 animals ± SE.

[a]P-value <0.05 vs. control group, [b]P-value <0.05 vs. ERC group, [c]P-value <0.05 vs. AH group.

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