Bulgarian Chemical Communications, Volume 57, Special Issue D (pp. 60-67) 2025 DOI:10.34049/bcc.57.D.MAEP8

Biological activity of the *Rosa alba* L. absolute against gentamicin-induced nephrotoxicity

S. R. Zlateva[1,4], V. N. Petrova-Tacheva[2], A. M. Dobreva[3], Z. L. Yaneva[4], E. D. Georgieva[5], G. D. Nikolova[1], Y. D. Karamalakova[1], K. V. Petkova-Parlapanska[1*]

[1]*Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, 11 Armeiska Str., 6000 Stara Zagora, Bulgaria*

[2]*Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, 6000 Stara Zagora, Bulgaria*

[3]*Institute for Roses and Aromatic Plants, Agricultural Academy, 49 Osvobojdenie Blvd., Kazanlak 6100, Bulgaria*

[4]*Department of Pharmacology, Animal Physiology, Biochemistry and Chemistry, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria*

[5]*Department of General and Clinical Pathology, Forensic Medicine, Deontology and Dermatovenerology, Medical Faculty, Trakia University, 6000 Stara Zagora, Bulgaria*

Revised: September 09, 2025

This study evaluated the nephroprotective effect of *Rosa alba* L. absolute (*R. alba*) against gentamicin (GM)-induced nephropathy in Balb/c mice (n = 24). The biological impact of *R. alba* absolute was evaluated using electron paramagnetic resonance (EPR) spectroscopy. The *in vivo* EPR assay measured the levels of nitric oxide (NO•), reactive oxygen species (ROS) products, and pro-oxidant malondialdehyde (MDA) in tissue homogenates from the kidneys of the experimental animals. Balb/c mice were divided into three groups (n = 6): Group 1: control group, on a common diet; Group 2: GM intoxicated (administered 200 mg kg^{-1} day^{-1}, 7 days); Group 3: Combination of GM (administered 200 mg kg^{-1} day^{-1}, 7 days) + *R. alba* absolute, administered per os (PO) (80 mg kg^{-1} day^{-1} of body weight). The antioxidant protective effects of white oil-bearing rose were confirmed by the modulation of ROS, NO•, and MDA. The *R. alba* absolute influence on antioxidant enzymes (glutathione (GSH), superoxide dismutase (SOD), catalase (CAT)) was monitored. The levels of antioxidant enzymes significantly decreased in the GM group, compared to Groups 1 and 2 ($p < 0.05$). In the treated groups, the application of *R. alba* absolute improved the studied parameters ($p < 0.05$). The results show that *R. alba* extract reduces the oxidative toxic effect of GM, and exhibits a potential nephroprotective effect against GM-induced renal injury.

Keywords: *Rosa alba* absolute, oxidative stress, gentamicin nephrotoxicity.

INTRODUCTION

Gentamicin (GM) is an aminoglycoside antibiotic with a pronounced antibiotic activity against Gram-negative bacteria. Clinically, the antibiotic is used in urinary tract infections [1], but its 7-day use is accompanied by increased toxicity and impaired metabolism, which impairs renal function [2]. The accumulation of gentamicin in the body is associated with its buildup within the Golgi apparatus, as well as in the endosomal and lysosomal compartments of proximal tubular cells in the kidneys. This intracellular accumulation is typically accompanied by distinct inflammatory responses in the affected renal tissues [1, 2]. Various hypotheses suggest that cytoplasmic accumulation of GM leads to the induction of oxidative stress (OS), followed by a decrease in mitochondrial activity, inflammation and fibrosis [3]. Prolonged therapy exacerbates OS by increasing the levels of short-lived reactive oxygen species (ROS/superoxide ion ($\bullet O_2^-$), hydrogen peroxide (H_2O_2)), nitrogen species (RNS/nitric oxide (•NO), peroxynitrite ($ONOO^-$)), and oxidized lipids [3, 4]. Redox signaling ROS and RNS are directly involved in metabolic regulation and adaptation to xenobiotic stress [5], and their intracellular imbalance is modulated by enzymatic and non-enzymatic systems (glutathione (GSH), uric acid, vitamin C), by natural ferrostatins (vitamin E), and by plant polyphenols [6-8]. GM therapy has been shown to reduce enzymatic efficiency, impair lipid peroxidation [9], and induce renal genotoxicity through increased OS. The potential of plant compounds containing polyphenols, flavonoids, rutin, quercetin, etc, leads to the mitigation of inflammatory processes in the body and modification of ROS and RNS production, especially in acute kidney injury. The white oil-bearing rose is a shrubby plant from the *Rosaceae* family, with significant antioxidant properties *in vitro*, not showing mutagenic effects

* To whom all correspondence should be sent:
E-mail: *kamelia.parlapanska@trakia-uni.bg*

[10]. Its aromatic products (essential oil, wastewater, or absolute) are characterized by active pharmacological applications due to phytocomponents such as: geraniol, nerol, citronellol, quercetin, isoquercetin, eugenol, methyleugenol, tannins, nonadecane, geranial acetate, squalene, kaempferol, quercetin, rutin, etc. [11]. In traditional medicine, *R. alba* L. is used to treat palpitations, headaches, colds, leprosy, stomatitis, bile, burning sensation, ophthalmology, bronchitis, rheumatism, diabetes, inflammation, microbial infection, uterine infection, stomach problems [12], etc. Nootropic effect, antimicrobial, and antidiabetic activity of *R. alba* L. have also been proven [13]. Previous research was focused on the redox-modulating capacity and antineoplastic activity of the *R. alba* L. wastewaters showed that *R. alba* L. exhibits the properties of a good heavy metal scavenger; it has a relatively stable anti-cytotoxic effect, and acts as a detoxifying agent under conditions of oxidative damage [10, 11]. Georgieva *et al*., [11] commented that *R. alba* L. demonstrated a high total polyphenol content (7.6 mg/mL) compared to quercetin. In addition, *R. alba* L. exhibits dose-dependent antioxidant activity in terms of scavenging DPPH• radicals, superoxide ion ($•O_2^-$) radicals, and $ABST^+$ concentrations, i.e., *R. alba* L. is a detoxifying agent under OS conditions [11]. In addition to wastewaters, *R. alba* extracts also registered antimutagenic effects and high antioxidant activity. The *R. alba* L. essential oil, when tested at concentrations of 250, 500, and 1000 μg/mL, showed weak cytotoxic and genotoxic effects on *H. vulgare* plant cells [14].

In this study, we investigated for the first time whether *R. alba* absolute exhibits protective therapeutic effects on GM-induced nephropathy in mouse models. This product is likely to prevent gentamicin-induced renal disease and inhibit oxidative damage by regulating the levels of ROS products, nitric oxide (NO•), and lipid peroxidation, and by restoring antioxidant enzymes (glutathione (GSH), superoxide dismutase (SOD), catalase (CAT)) in kidney tissue homogenates and blood serum of Balb/c mice.

EXPERIMENTAL

Plant material

R. alba absolute was provided by the Institute of Rose and Essential Oil Crops - Kazanlak. Briefly: fresh rose blossom from *R. alba* L. (500 g of whole blossoms - without external moisture) is subjected to a three-fold extraction with n-hexane, by maceration under static conditions in a scheme of battery-connected extractors. The duration of the extractions is 5 min - 30 min - 15 min, respectively. This model allows for complete extraction of aromatic substances with minimal solvent consumption. The obtained extracts are combined, filtered and evaporated under vacuum at 40 °C until the hexane is completely removed to obtain a specific - yellow-orange, waxy and paraffin-like semi-solid substance. The product thus obtained is dissolved in 96 % ethanol, with continuous shaking and gentle heating. The resulting fraction is cooled at room conditions, then stored for 24 h at sub-zero temperatures (-20 °C). It is filtered cold (laboratory system with funnel, suction flask and water pump) to remove the waxes. This manipulation is performed three times, until the waxes are completely exhausted. The filtrates are combined, dehydrated with Na_2SO_4 and subjected to vacuum evaporation to remove the ethanol. The final product obtained is the so-called absolute – a yellow-orange viscous liquid, with a typical smell of rose.

The authenticity of the rose species was confirmed by Trakia University, Stara Zagora, Bulgaria, and the voucher specimens were deposited at the IBER-BAS herbarium with the following numbers: *R. alba* - SOM 178484.

Gas chromatography analysis

The chemical composition of the absolute was determined by gas chromatography. GC system (Agilent 7820A) coupled with a flame ionization detector was used. The capillary column EconoCap™ EC™-5 (30 m × 0.32 mm × 0.25 μm film of 5 % phenyl, 95 % methylpolysiloxane) was used. The oven temperature program and detector and injector temperatures were set as described in BDS 17381 - 96 "Concret of roses. Rose absolute". Hydrogen was used at a flow rate of 1.1 mL min^{-1} as carrier gas. The calculation of the relative percentages was performed by GC-FID peak area without a correction factor. The identification of constituents was performed by comparing the retention indices and matching with co-injections of authentic compounds.

Animals

Balb/c mice (n=24; 35 – 37.8 g; 9 weeks old, Neurobiology Institute, Slivnitsa, Bulgaria), used in this study, were kept in the animal care facility by the Bioethics Committee, TrU, Stara Zagora, Bulgaria. The following protocol was followed throughout the experiment: six animals in a polycarbonate cage, temperature 21 °C, relative humidity 50 %, dark/light cycle photoperiod from 7 to 19 h, unhindered access to the usual diet and

fluids for the maintenance of laboratory animals, filtered water (pH = 5.5; *ad libitum*). All procedures were in accordance with the requirements of the Animal Ethics Committee, with a license (317/6000-0333/09.12.2021) following Directive 2010/63/EU on the animals' protection used for experimental and other scientific work.

Experimental procedure

The mouse model of GM-induced nephrotoxicity was developed by daily intraperitoneal injection of 200 mg $kg^{-1}day^{-1}$ for 7 consecutive days [15, 16]. GM induction causes oxidative stress disorders and generates a cascade of redox-homeostatic imbalances directly affecting the renal tubules.

R. alba absolute has shown pharmacological properties at doses ranging from 250 to 1000 μg mL^{-1} [11]. Its pharmacological effects may be different depending on the concentrations of flavonoids and polyphenolic components. Based on preliminary *in vitro* studies [11, 14] of antioxidant activity and cytoprotective effect, 80 mg kg^{-1} *R. alba* absolute was used in the present study. *R. alba* absolute was mixed in isotonic NaCl solution (0.9 %) and homogenized for 15 min before injection.

Animals were randomly divided into four groups (n = 6), according to (1) controls - basal diet (19.6 % protein, 4.03 % fat, 6.89 % fiber, 10.71 % moisture; 8.97 % ash), injected intraperitoneally with 1 mL of isotonic NaCl solution (0.9 %); (2) *R. alba* absolute administered orally (PO) only (80 mg kg^{-1} day^{-1} body weight); (3) GM only - injected IP (200 mg kg^{-1} day^{-1} body weight) to induce acute nephrotoxicity; (4) GM + *R. alba* absolute therapy - GM was injected IP (200 mg kg^{-1} body weight), and *R. alba* absolute (80 mg kg^{-1} day^{-1} body weight) was administered 2 h after GM injection (Fig. 1).

The *R. alba* administration at a dose of 80 mg kg^{-1} day^{-1} (body weight) improved the vitality of the animals, similar to the control group. The physiological state and behavior of the animals were monitored daily. Anxiety, weakness, muscle twitching, vomiting, abdominal pain, shortness of breath, blood in urine and feces, and a percentage mortality (0 %) were not recorded.

Twenty-four hours after the last dose, mice were weighed and anesthetized by IP injection (Nembutal, 50 mg kg^{-1}). Fresh kidney tissues were chilled, homogenized, and analyzed. The mice's kidneys were weighed, and the right kidney was placed in ice-cold 0.05 M PBS (pH= 7.5; 4°C), homogenized individually, and analyzed.

Lipid peroxidation and endogenous antioxidant activity

Lipid peroxidation in kidney tissue was assessed by the method of Plaser *et al.* [17], against equivalent concentrations of malondialdehyde (MDA) in μmol/mg protein (TERMO Sci., RS232C, USA).

Renal catalase (CAT) activity was assessed by the Aebi method [18], at an absorbance of 240 nm. The activity of cellular nephritic superoxide dismutase (SOD) was analyzed by Sun *et al.* [19], at 420 nm absorption. Glutathione (GSH) levels in kidney homogenates were assessed using the method of Akerboom and Sies, 1981 [20]. Reduced GSH was assessed by continuous DTNB reduction, expressed as nanomoles of GSH per ml of protein. A blank without blood/tissue homogenate was prepared similarly and absorbance was recorded at 412 nm.

Oxidative stress analysis in renal tissue

ROS production: A total of 100 μL of homogenized kidney tissue was mixed with 900 μL (50 mM) of N-*tert*-butyl-alpha-phenylnitrone (PBN) dissolved in DMSO. The mixture was centrifuged at 4000 × *g*, 10 min at 4 °C, by [21].

The nitric oxide (•NO) generation relative to the spin–adduct formed between the spintrap carboxy 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl (CPTIO.K) and •NO in kidney tissue were based on established EPR methods [22, 23]. Briefly, 50 μM CPTIO.K was dissolved in a mixture of 50 mM Tris (pH = 7.5), and DMSO (9:1) and was centrifuged at 4000× *g* for 10 min at 4 °C. Then, 100 μL kidney samples were mixed in 100 μL of CPTIO.K, and spin–adducts were recorded.

The superoxide ($\bullet O_2^-$) concentration in kidney tissue was determined relative to the spin–adduct formed using the spin-trap CMH (1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine), based on methods [24, 25]. For this purpose, 30 μL of kidney tissue was activated in 30 μL of CMH (1:1) on an ice bath, and after 5 min incubation, it was ready for use.

Spectral EPR analyses were performed with fivefold measurement of EPR spectra, with the following characteristics: 3503–3515 G center field; 6.42–20.00 mW microwave power; 5–10 G modulation per sample, and the results were presented in arbitrary units (a.u.). Spectral processing was performed using Bruker WIN-EPR accessed 2019 and *Simfonia*, software version 1.2.

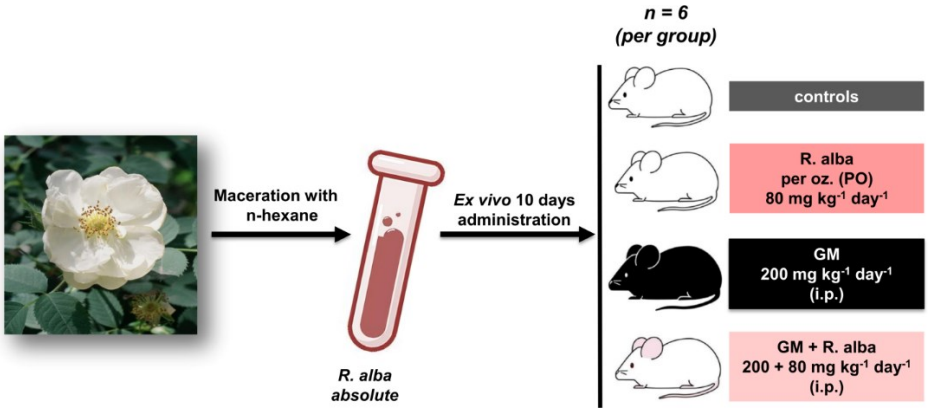

Fig. 1. Experimental protocol design of *R. alba* absolute *in vivo* acute administration.

Statistical analysis

Statistical analysis was performed with Statistica 8, StaSoft, Inc. (Madrid, Spain), and the results were expressed as means ± SE. All data were expressed as means ± SE and obtained by one-way ANOVA, and in the LSD post hoc test, $p > 0.05$ was considered statistically significant. LSD post hoc tests were used to define which groups were different from each other.

RESULTS AND DISCUSSION

In addition to the economically important essential rose oil from the Bulgarian varieties *Rosa alba* L., *Rosa damascena* Mill., *Rosa gallica* L., and *Rosa centifolia* L., other residual products (concrete, absolute, rose water) are generated during production, which have not yet been fully investigated, and interest in studying their use, especially in cellular and animal models [10-13, 14] is growing.

R. alba absolute chemical compositions

R. alba concrete is primarily used for the preparation of *R. alba* absolute. Chromatographic analysis of the present *R. alba* absolute revealed various acyclic, related monoterpene alcohols, while sesquiterpene hydrocarbons and sesquiterpene oxygenates were present in small concentrations (Table 1).

As shown in Table 1, the highest monoterpene alcohols included aryl-alcohols (phenylethyl alcohol, 16.2 %), geraniol (9.89 %), and citronellol/nerol (6.17 %). The relatively absent content of the phenylpropanoid eugenol (0.02 %) practically confirms the low toxicity and lack of carcinogenicity (Opinion of the Scientific Committee on Food on methyl eugenol, 2001); Rusanov *et al.* [26] of *R. alba* absolute. Geraniol exhibits a robust inhibitory effect on CCL_4-induced toxicity and normalizes the architecture of renal corpuscles, with their glomeruli and proximal and distal renal tubules [27].

Table 1. Characteristics of *R. alba* absolute chemical compounds in GC-FID analysis.

Rosa type	No	Compounds	Relative percent, (%)
R. alba absolute	1	Ethanol (C_2H_6O)	0.86
	2	Benzyl alcohol (C_7H_8O)	1.58
	3	Phenylethyl-alcohol (C_8H_{10})	16.2
	4	Citronellol/Nerol ($C_{10}H_{20}$)	6.17
	5	Geraniol ($C_{10}H_{18}O$)	9.89
	6	Eugenol ($C_{12}H_{20}O_2$)	0.02
	7	Methyleugenol ($C_{11}H_{14}O_2$)	0.01
	8	trans-β-Caryophyllene ($C_{15}H_{24}$)	1.31
	9	Heptadecane ($C_{17}H_{36}$)	0.19
	10	Nonadecene ($C_{19}H_{38}$)	3.00
	11	Nonadecane ($C_{19}H_{40}$)	6.69

*Results are shown as mean ± RSD (n=2)

In addition, the antioxidant and anti-inflammatory properties of citronellol/nerol reduce the oxidative damage to the kidneys caused by GM-induced nephrotoxicity [28]. GC-FID analysis also revealed a component predominance of trans-β-caryophyllene/caryophyllene. The natural bicyclic sesquiterpene has a unique structure involving a cyclobutane ring and a trans-double bond in a 9-membered ring. Scandiffio *et al.* [29] commented on the modulatory and pharmacological effects of the sesquiterpene hydrocarbon (E)-β-caryophyllene (BCP) in organs such as liver and brain [30, 31]. In addition, BCP exerts therapeutic effects as an antioxidant agent and anti-inflammatory protector [32] in the kidneys [28, 33].

R. alba absolute ameliorate lipid peroxidation against GM-induced acute inflammation

Megalin and tubulin endocytosis promote GM deposition in proximal renal tubules. This alters lysosomal aggregation, phospholipid metabolism, and mitochondrial toxicity [3, 13]. Consequently,

OS promotes the ROS/RNS accumulation and additional acute oxidative stress [30] damages. At the mitochondrial level, ROS/RNS modulation occurs through metabolic factors, proinflammatory deactivators, as well as endogenous and exogenous antioxidant deactivation of organelles [6, 7]. The biologically active components, monoterpene alcohols, sesquiterpene hydrocarbons, and sesquiterpene oxygen compounds, contribute to nephron protection due to antioxidant and anti-inflammatory mobilization [27, 28, 32, 33].

Based on these facts, we aimed to investigate the protective effect of *R. alba* absolute extract against acute kidney injury induced by GM therapy. Furthermore, we investigated the antioxidant regulatory mechanism of *R. alba* absolute by maintaining endo-exogenous deactivation, redox homeostatic imbalance and lipid peroxidation.

The study of the effects after 7-days of GM administration on renal enzyme activities and the lipid peroxidation degree is presented in Figure 1. Renal SOD (1.296 ± 0.37 *vs*. 4.78 ± 0.89 U/gHb, $p < 0.005$; Figure 2A), CAT (1.66 ± 0.17 *vs*. 3.85 ± 0.05 U/gPr, $p < 0.05$; Figure 2B), and GSH (228.40 ± 33.4 *vs*. 364.5 ± 17.39 µmol/mL, $p < 0.004$, Figure 2C) decreased statistically significantly compared to controls, and indicating acute oxidative impairment of renal functions. However, GM significantly increased MDA concentration in kidneys (5.99 ± 0.87 vs. 3.044 ± 0.287 µmol/mL, $p < 0.05$; Figure 2D) compared with controls.

GM-treated mice showed almost twofold decreased SOD, CAT enzyme activity and partial depletion of GSH activity. This fact confirms that GM stimulates the mitochondrial respiratory chain to accumulate H_2O_2, i.e. $\bullet O_2^-$, $^1O_2$, HO•, OH^-, NO•, and peroxy (R/ $ONOO^-$) radicals in the kidney tissue. GM induces acute renal inflammation and oxidative damage accumulation in the renal tubules and glomeruli. These OS damages are probably a consequence of the inflammatory cells activation and physiologically impaired redox modulation, which increases lipid peroxidation [7]. The reported twofold increase in MDA concentrations after 7 days of GM accumulation is a sign of activated nephrotoxicity. The presented results are in accordance with previous studies [3, 7-9]. Conversely, combined administration of *R. alba* absolute at a dose of 80 mg kg^{-1} showed statistically significant toxicity inhibition and modulation of GM-induced renal disorders. These processes occur simultaneously with a statistically significant increase in endo-exogenous enzymatic protection.

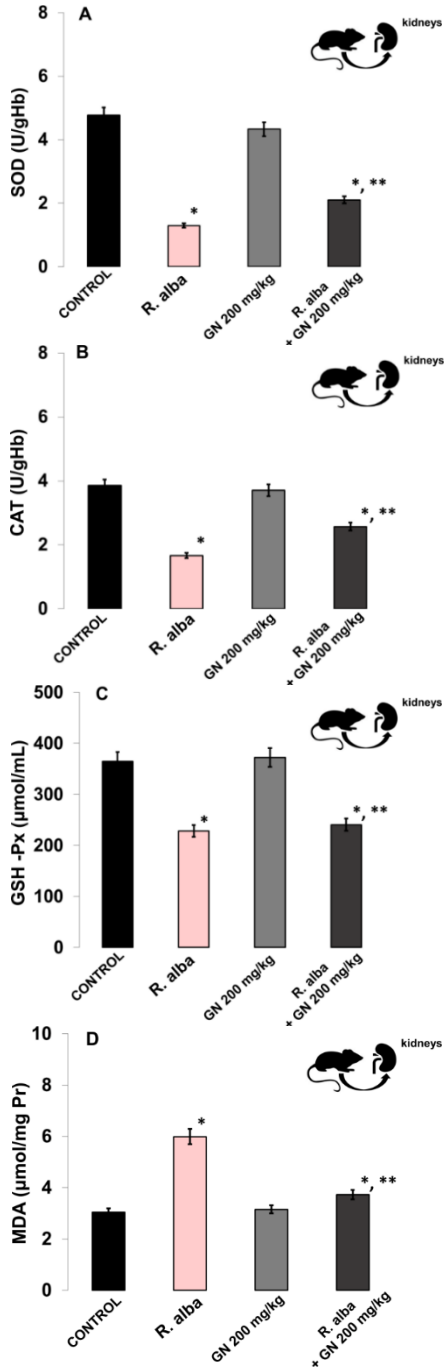

Figure 2. *R. alba* absolute effects on GM-induced nephritic enzyme activities and lipid peroxidation in tested groups: controls, *R. alba* absolute treated mice, GM-treated mice and *R. alba* absolute + GM-treated mice: (A) superoxide dismutase (SOD, U/gPr), (B) catalase (CAT, U/gPr), (C) reduced glutathione (GSH; µmol/ml), (D) malondialdehyde concentration (MDA, µmol/mgPr). The results are presented as mean ± SD (n=6). One-way ANOVA with multiple comparisons using Student's t-test was used to determine significant differences to (*) $p < 0.05$ *vs*. controls, (**) $p < 0.005$ *vs*. GM-treated mice.

R. alba absolute at a dose of 80 mg kg^{-1} is probably able to deactivate the H_2O_2, $\bullet O_2^-$, HO•, OH^- radical accumulation, i.e. reduce and

deactivate the renal OS damages. Statistically significant reduced activity of SOD (2.106 ± 0.31 *vs*. 4.78 ± 0.89 U/gHb, $p < 0.005$; Figure 2A), CAT (2.573 ± 0.22 *vs*. 3.855 ± 0.05 U/gPr, $p < 0.05$; Figure 2B), GSH (243.60 ± 31.9 *vs*. 364.5 ± 17.39 μmol/mL, $p < 0.004$, Figure 2C) and MDA (3.725 ± 0.4 *vs*. 5.99 ± 0.96 μmol/mL, $p < 0.05$, Figure 2D) was recorded, compared to controls (Figures 2A-D).

Antioxidantly, *R. alba* absolute (80 mg kg^{-1}), both alone and in combination GM + *R. alba* absolute, reverses hyperpolarization and restores the endogenous/exogenous redox homeostasis. This occurs after a possible reversal of the respiratory chain role, compared to GM accumulation, and to controls. *R. alba* L. (wastewaters, extracts, etc.) has been reported to possess metal-chelating properties [11]. *R. alba* L. sequesters copper (II) to a non-redox-active form, i.e. prevents oxidative damage to cell membranes and increases cell survival. Reduced cellular oxidation is accompanied by reduced initiation of •OH radicals [34, 35] and direct destruction of membrane lipids [7, 11]. Georgieva et. al. [11] drew attention to the fact that antioxidants such as *R. alba* L., have the ability to chelate and reduce iron (III) ions, which allows for the potential maintenance of membrane-metal homeostasis. In addition, the chelation and reduction of iron (III) ions supported by *R. alba* absolute protectively control ferroptotic processes by suppressing toxic ROS/RNS. Therefore, *R. alba* absolute restores the SOD, CAT enzymatic activity, by further activating GSH levels, which leads to H_2O_2 detoxification and increased cellular reduction of lipid peroxides (L-OOH). Moreover, *R. alba* absolute contains high polyphenol levels (7.6 mg mL^{-1}) [10, 11] and acyclic monoterpene alcohols (geraniol (9.89 %); citronellol/nerol (6.7 %)). These agents have been shown to be effective in protecting against GM-mediated and doxorubicin-mediated renal injuries and fibrosis development, due to potent ihibition of residual lipid peroxidation [28, 36].

R. alba absolute ameliorated ROS, NO• stress levels against GM-induced acute inflammation

Cell-permeable spin probes (based on aminoxyl, nitronyl nitroxide, or hydroxylamine (CPTIO.K, CMH) radicals) allow for the accurate determination of ROS/ RNS and directly reflect differences in redox status, *in vivo* [37]. The spin probes are easily reduced to the corresponding diamagnetic forms and act as catalysts for the destruction of renal NO• to the formation of the imino-nitroxide radical, or the dimutation of renal $•O_2^-$ to H_2O_2 and molecular oxygen (O_2) [38, 39].

To confirm the protective role of *R. alba* absolute against GM-mediated oxidative renal disorders and inflammatory processes, the redox-modulated activity was investigated.

GM administration significantly increased the ROS products (2.855 ± 0.31 vs. 1.09 ± 0.08 a.u., $p < 0.005$; Figure 3) and NO• levels (56.61 ± 5.31 *vs*. 17.88 ± 1.66 a.u., $p < 0.002$; Figure 3) in kidney homogenates, compared to controls. The strong antioxidant protection of *R. alba* absolute after GM induction was reflected in the reduced toxic ROS production (1.456 ± 0.19 *vs*. 2.855 ± 0.31 au, $p < 0.05$) and in cellularly absorbed NO• concentrations (39.61 ± 3.001 *vs*. 56.83 ± 5.31 au, $p < 0.05$) in kidneys. These findings highlight the protective effects of the combined treatment of GM+ *R. alba* absolute on renal OS, compared to mice treated with GM alone. It should be noted that *R. alba* absolute (7 days) likely alleviated oxidative lesions in renal proximal tubule cells through redox dismutation of renal ROS production and NO• radicals to nontoxic molecular oxygen (O_2), compared to the GM-treated group. Furthermore, ROS/ RNS dismutation activated the enzymatic minimization of inflammatory responses.

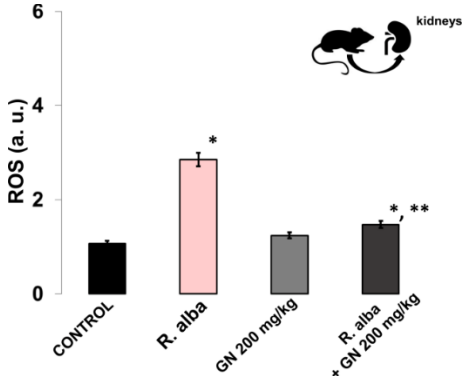

Figure 3. *R. alba* absolute effects on GM-induced nephrotoxicity and oxidative changes in ROS production. The radicals were scavenged in triplicate by EPR spectroscopy using *Win-EPR* and *Simfonia* software and expressed in arbitrary units (a.u.). The results are presented as mean ± SD (n = 6). One-way ANOVA with Student's t-test was used to determine statistically significant differences in relation to *$p < 0.05$ *vs*. controls and **$p < 0.005$ *vs*. GM-treated mice.

R. alba absolute containing phenylethyl alcohol, citronellol/nerol, geraniol, trans-β-caryophyllene prevents the transformation of $•O_2^-$ into toxic $ONOO^-$ and •OH radicals. Simultaneously, the active antioxidant components restore the NO bioavailability as a signaling molecule, regulating renal blood flow. Protection with *R. alba* absolute

for 7-days showed significant catalysis of ROS production and RNS elevation by reducing oxidative stress damage, followed by activation of the mitochondrial respiratory chain [40, 41]. Also, antioxiantly *R. alba* absolute corrects GM accumulation by directly reducing collagen deposition, suppressing vasoconstriction, and antioxidant-mediated renal fibrosis [41, 42]. Verma *et al.,* [42] reported that *R. alba* L. (oil; rose water etc.) effectively controlled lipid profile abnormalities and preserved normal renal architecture and renal function.

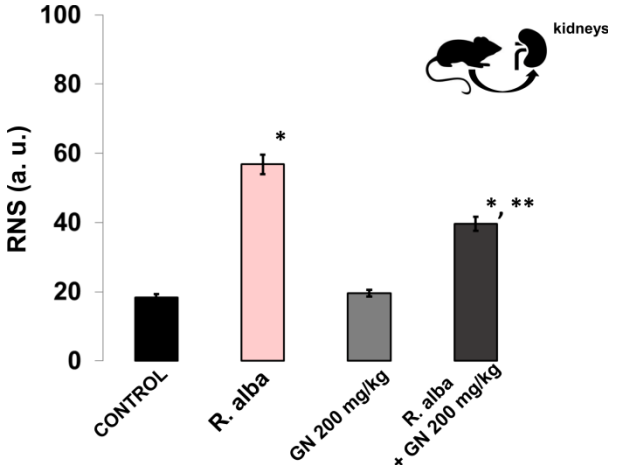

Figure 4. The *R. alba* absolute effects on GM-induced nephrotoxicity and oxidative changes in nitric radicals (NO•). The radicals were scavenged in triplicate by EPR spectroscopy using Win-EPR and Sim-Fonia software and expressed in arbitrary units (a.u.). The results are presented as mean ± SD (n = 6). One-way ANOVA with Student's t-test was used to determine statistically significant differences in relation to *p < 0.05 *vs.* controls and **p < 0.005 *vs.* GM-treated mice.

In line with this, Ilieva *et al.* [10] demonstrated that Bulgarian oil-bearing roses *R. alba* L., *Rosa damascena* Mill., *R. centifolia* L., and *R. gallica* L. exhibit significant antioxidant activity, ROS/RNS-protecting effects, and toxicological safety against non-tumorigenic human embryonic kidney cells (HEK-293) and mouse fibroblasts (CCL-1). *R. alba* absolute, through its antioxidant activities, deactivates free radicals in the presence of reduced ferric and copper ions. These processes, in turn, catalyze the overproduction of toxic •OH radicals and inhibit ferroptosis [10, 11, 36, 43]. Furthermore, the active citronellol/nerol, geraniol, and trans-β-caryophyllene in *R. alba* absolute likely promote renal blood flow, increasing glomerular filtration and remodulating the NF-kB pathway and Th2 immune response [36, 43, 44].

***Study limitations**: Due to ethical considerations, the number of animals studied per group was six, which is sufficient to reveal the most significant differences, but may be insufficient to establish additional correlations between some parameters. In the present study, in vivo GM activity was not investigated by R. alba absolute co-administration, which would also reveal additional information about the antimicrobial properties of the combination therapy. Further studies are needed to profile the R. alba absolute toxicity, including dose-response analyses, histopathological validation, and identification of possible doses with a beneficial effect on renal intoxication in longer-term use.*

CONCLUSIONS

The present study highlights the antioxidant activity and anti-inflammatory potential of *R. alba* absolute (80 mg mL^{-1}) under GM-induced nephrotoxicity. The antioxidant protection is mediated by a reduction in lipid peroxidation and restoration of enzyme activities. *R. alba* absolute directly related to the redox modulation of free radicals, which facilitate mitochondrial antioxidants and anti-inflammatory properties, without compromising the efficacy of bactericidal therapy.

***Acknowledgement:** This research was supported by the Bulgarian Ministry of Education and Science (MES) in the framework of the Bulgarian National Recovery and Resilience Plan, Component \u201CInnovative Bulgaria\u201D the Project \u2116 BG-RRP-2.004-0006-C02 \u201C Development of research and innovation at Trakia University in service of health and sustainable well-being\u201D; and supported by the University Projects № 10/2022 and № 2/2025, Medical Faculty, Trakia University, Bulgaria.*

***Conflicts of Interest:** The authors declare no conflicts of interest.*

REFERENCES

1. M. Mahi-Birjand, S. Yaghoubi, M. Abdollahpour-Alitappeh, Z. Keshtkaran, N. Bagheri, A. Pirouzi, I. Karimzadeh, *Expert Opin. Drug Saf.,* **19**, 167 (2020).
2. M. Balgradean, E. Cinteza, F. Filipoiu, V. Jinga, *Farmacia,* **61**, 772 (2013).
3. R. Sandoval, B. Molitoris, *Am. J. Physiol. Renal. Physiol*., **286**, 617 (2004).
4. H. Sies, D. Jones, *Nat. Rev. Mol. Cell Biol*., **21**, 363 (2020).
5. P. Singh, K. Aprajita, J.G. Kapuganti, *Physiol. Plant.,* **2**, 174 (2022).
6. C. A. Juan, J. Pérez de la Lastra, F. Plou, E. Pérez-Lebeña, *Int. J. Mol. Sci.*, **22**, 4642 (2021).
7. F. Collin, *Int. J. Mol. Sci.,* **20**, 2407 (2019).

8. N. Vilhelmova-Ilieva, Z. Petrova, E. Georgieva, M. TzvetanovaTrepechova, M. Mileva, *Life,* **12**, 1088 (2022).
9. R. Ungur, I. Borda, R. Codea, V. Ciortea, B. Năsui, S. Muste, G. Martiș, *Materials,* **15**, 772 (2022).
10. Y. Ilieva, L. Dimitrova, A. Georgieva, N. Vilhelmova-Ilieva, M. Zaharieva, Z. Kokanova-Nedialkova, A. Dobreva, P. Nedialkov, V. Kussovski, A. Kroumov, *Plants,* **11**, 1073 (2022).
11. A. Georgieva, Y. Ilieva, Z. Kokanova-Nedialkova, M. Zaharieva, P. Nedialkov, A. Dobreva, A. Kroumov, *Antioxidants*, **10**, 1615 (2021).
12. S. Alom, F. Ali, R. Bezbaruah, B. Kakoti, *World Journal of Pharmaceutical Research*, **10**, 798 (2021).
13. R. Dadhwal, R. Banerjee, *South African Journal of Botany*, **158**, 98 (2023).
14. M. Mileva, Y. Ilieva, G. Jovtchev, S. Gateva, M. Zaharieva, A. Georgieva, H. Najdenski, *Biomolecules*, **11**, 127 (2021).
15. F. Collin, *Int. J. Mol. Sci.*, **20**, 2407 (2019).
16. N. Zeima, A. EL-Gawish, *Egypt. J. Nutr. Health*, **16**, 35, (2021).
17. Z. Placer, L. Cushman, B. Johnson, *Anal. Biochem.*, **16**, 359 (1966).
18. H. Aebi, *Academic Press: Cambridge, MA, USA,* **105**, 121 (1984).
19. Y. Sun, L. Oberley, Y. Li, *Clin. Chem.*, **34,** 497 (1988).
20. T. Akerboom, M. Bilzer, H. Sies, *J. Biol. Chem.* **257**, 4248 (1982).
21. H. Shi, Y. Sui, X. Wang, Y. Luo, L. Ji, *Comp. Biochem. Phys. Part C Toxicol. Pharmacol.*, **140**, 115 (2005).
22. T. Yoshioka, N. Iwamoto, K. Lto, *J. Am. Soc. Nephrol.*, **7**, 961 (1996).
23. K. Yokoyama, K. Hashiba, H. Wakabayashi, K. Hashimoto, K. Saton, T. Kurihara, N. Motohashi, H. Sakagami, *Anticancer Res.*, **24**, 3917 (2004).
24. J. Gielis, G. Boulet, J. Briedé, T. Horemans, T. Debergh, M. Kussé, P. Van Schil, *Eur. J. Cardiothorac. Surg.*, **48**, 622 (2015).
25. S. Perrone, A. Santacroce, M. Longini, F. Proietti, F. Bazzini, G. Buonocore, *Oxidative Med. Cell. Longev.*, 7483062 (2018)..
26. K. Rusanov, N. Kovacheva, M. Rusanova, I. Atanassov, *Food Chem.*, **129**, 1851, (2011).
27. S. Alzahrani, G. Bekhet, R. Ammar, B. Abdallah, E. Ali, S. Al-Ramadan, D. Althumairy, P. Rajendran, *Saudi Journal of Medicine & Medical Sciences*, **12**, 17 (2024).
28. S. Rahimi Monfared, A. Valibeik, N. Tavakoli Dastjerd, L. Jafaripour, A. Jafarian, M. Nabi Moradi, H. Ahmadvand, *Molecular biology reports*, **51**, 382 (2024).
29. R. Scandiffio, F. Geddo, E. Cottone, G. Querio, S. Antoniotti, M. Gallo, M. Maffei, P. *Nutrients*, **12**, 327 (2020).
30. M. Calleja, J. Vieites, T. Montero-Meterdez, M. Torres, M. Faus, A. Gil, A. Suárez, *Br. J. Nutr.*, 109 (2013).
31. H. Javed, S. Azimullah, M. Haque, S. Ojha, *Front. Neurosci.,***10**, 321 (2016).
32. G. Picciolo, G. Pallio, D. Altavilla, M. Vaccaro, G. Oteri, N. Irrera, F. Squadrito, *Biomedicines*., **8**, 164 (2020).
33. B. Horváth, P. Mukhopadhyay, M. Kechrid, V. Patel, G. Tanchian, D. Wink, J. Gertsch, *Free Radic. Biol. Med.*, **52**, 1325 (2012).
34. R. Prior, X. Wu, K. Schaich, *J. Agric. Food Chem.*, **53**, 4290 (2005).
35. J. Gutteridge, B. Halliwell, *Biochem. Biophys. Res. Commun.*, **502**, 183 (2018).
36. A. Abdullah, N. Ali, M. Alharbi, F. Alqahtani, F. Alasmari, D. Almoqbel, M. AlSwayyed, *Nutrients*, **14**, 1620 (2022).
37. M. Abo, E. Weerapana, *Antioxid. Redox Signal.,* **30**, 1369 (2019).
38. N. Babić, F. Peyrot, *Magnetochemistry*, **5**, 13, (2019).
39. M. Lewandowski, K. Gwozdzinski, *Int. J. Mol. Sci.*, **18**, 2490 (2017).
40. P. Chaudhary, P. Janmeda, A. Docea, B. Yeskaliyeva, A. Abdull Razis, B. Modu, D. Calina, J. Sharifi-Rad, *Front. Chem.*, **11**, 1158198 (2023).
41. S. Kang, T. Chen, Z. Hao, X. Yang, M. Wang, Z. Zhang, S. Hao, F. Lang, H. Hao, *Molecules*, **27**, 6209 (2022).
42. A. Verma, R. Srivastava, P. Sonar, R. Yadav, *The Journal of Phytopharmacology*, **13**, 167 (2024).
43. Z. Jin, F. Mollica, Y. Huang, S. Guernelli, A. Baschieri, C. Diquigiovanni, N. Rizzardi, F. Valenti, L. Pincigher, *Chemistry–A European Journal*, **30**, (2024).
44. M Jamal, S. Kathem, *Naunyn-Schmiedeberg's Archives of Pharmacology,* **398**, (2025).