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Phytochemical profile and hepatoprotective effects of *Crotalaria vialattei* against antituberculosis drug-induced liver injury

Kaouther Zouioueche[1,2,*], Meriem Laraba[1,2], Afifa Bensegueni[3], Iman Ramli[4], Leila Hammoud[1], Ramazan Erenler[5], İlyas Yıldız[6], Imene Messaoud Nacer[7], Aya Berkane[1,8], Chawki Bensouici[9], Fadila Benayache[1], Samir Benayache[1], and Djamila Zama[1,2]

[1]*Research Unit, Valorization of Natural Resources, Bioactive Molecules, and Physicochemical and Biological Analyses (VARENBIOMOL), University of Constantine 1 Frères Mentouri*, Ain El-Bey Road, 25000, Constantine, Algeria

[2]*Department of Animal Biology, Faculty of Natural and Life Sciences, University of Constantine 1 Frères Mentouri*, Aïn El Bey Road, 25000, Constantine, Algeria

[3]*Department of Anatomic Pathology, Faculty of Medicine, University Salah Boubnider Constantine 3*, Constantine, Algeria

[4]*Applied Biochemisty laboratory, University of Constantine 1 Frères Mentouri*, Ain El Bey Road, 25000, Constantine, Algeria

[5]*Department of Chemistry, Faculty of Arts and Sciences, Tokat Gaziosmanpasa University*, 60240 Tokat, Türkiye

[6]*Department of Molecular Biology and Genetics, Department of Biotechnology, Institute of Science, Tokat Gaziosmanpaşa University*, 60240 Tokat, Türkiye

[7]*Laboratory of Biotechnology, Environment and Health, University of Blida 1*, Blida, Algeria

[8]*Department of Biochemistry and Cellular and Molecular Biology, Faculty of Natural and Life Sciences, University of Constantine 1 Frères Mentouri*, Aïn El Bey Road, 25000, Constantine, Algeria

[9]*Biotechnology Research Center (CRBT)*, Ali Mendjeli New Town UV 03, Constantine 25000, Algeria

***Corresponding author**: kaouther.zouioueche@doc.umc.edu.dz

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Abstract: Tuberculosis is a global public health concern, and its treatment is frequently associated with hepatotoxic side effects. Phytotherapy represents a promising complementary approach. This study aimed to characterize the phenolic profile of the *n*-butanol extract of *Crotalaria vialattei* (BCV), evaluate its antioxidant capacity, and assess its hepatoprotective effects against liver damage induced by a fixed-dose antituberculosis drug combination containing rifampin, isoniazid, and pyrazinamide (RHZ). Phytochemical analysis revealed a phenolic-rich extract, identifying 17 polyphenolic compounds. BCV exhibited measurable antioxidant activity *in vitro. In vivo*, oral administration of RHZ (rifampin 150 mg/kg, isoniazid 75 mg/kg, and pyrazinamide 400 mg/kg) induced marked alterations in hepatic biochemical markers, lipid profile, oxidative status, and liver histoarchitecture. BCV treatment significantly attenuated these changes by improving liver enzyme activities, restoring oxidative balance, and preserving liver architecture. Overall, the BCV extract demonstrates antioxidant-associated hepatoprotective potential against RHZ-induced liver injury and may represent a promising complementary strategy to reduce antituberculosis drug-related hepatotoxicity.

Keywords: *Crotalaria vialattei,* RHZ, polyphenols, antioxidant activity, hepatoprotective effect

INTRODUCTION

Tuberculosis is a major respiratory infectious disease caused by *Mycobacterium tuberculosis* (Mtb), an intracellular human pathogen. Despite the availability of effective chemotherapy, tuberculosis remains a leading cause of mortality worldwide. In 2016, it was reported as the primary cause of death due to a single infectious agent, accounting for approximately 1.3 million deaths among HIV-negative individuals [1].

The standard first-line treatment for tuberculosis relies on a combination of rifampicin, isoniazid, and pyrazinamide (RHZ), particularly during the

intensive phase of therapy. Although this regimen is highly effective in controlling infection, its clinical use is frequently limited by hepatotoxic adverse effects, which are a major cause of treatment interruption and therapeutic failure [2]. RHZ-induced hepatotoxicity is widely attributed to hepatic drug metabolism, leading to oxidative stress, lipid peroxidation, and functional impairment of hepatocytes [3]. Isoniazid (INH) and rifampin (RIF) are extensively metabolized in the liver, and their combined administration has been reported to increase the risk of liver injury compared to monotherapy. Pyrazinamide (PZA) is considered the most hepatotoxic component of the regimen, particularly when administered in combination with other first-line drugs, further amplifying the risk of liver dysfunction [4]. Consequently, antituberculosis drug-induced hepatotoxicity remains a major clinical challenge, highlighting the need for complementary strategies aimed at preserving hepatic function without compromising therapeutic efficacy.

Medicinal plants have long been used in traditional medicine for the management of liver disorders and are increasingly studied as complementary therapeutic approaches. Numerous experimental studies have demonstrated that plant-derived extracts and their bioactive constituents can exert hepatoprotective effects against drug-induced liver injury, often through antioxidant and cytoprotective mechanisms [5].

The genus *Crotalaria L.* (within the tribe Crotalarieae of the Fabaceae family) is one of the largest legume genera, comprising approximately 702 species. Most of these species (around 400) exist in tropical and subtropical Africa. *Crotalaria* species are readily identifiable by their yellow, whitish, purplish, or bluish flowers. Their leaves are typically simple or composed of 1 to 3 leaflets, arranged alternately, and vary in shape from lanceolate to obovate [6]. Plants belonging to this genus are known to synthesize a wide array of biologically active compounds, including alkaloids, flavonoids, phenolic acids, and saponins [7]. These species are medicinally important, exhibiting antispasmodic, antioxidant, immunomodulatory, antimicrobial, anticancer, anti-inflammatory, antifungal, antiulcer, antitumor, and hepatoprotective activities [8]. In particular, phenolic compounds are noteworthy plant-derived secondary metabolites that act as antioxidants and may be used as supplements to activate the body's natural defensive protein systems, thereby contributing to the maintenance of a balanced redox state essential for cellular homeostasis and enzymatic activity [9].

Crotalaria vialattei remains an underexplored species, and data regarding its potential protective effects against drug-induced hepatotoxicity are currently scarce. Therefore, the present study aimed to evaluate the antioxidant and hepatoprotective potential of the *n*-butanol extract of *C. vialattei* (BCV) against RHZ-induced liver injury in a rat model. In addition, the polyphenolic profile of BCV was characterized to relate its phytochemical composition to the observed biological effects.

MATERIALS AND METHODS

Ethics statement

The experimental protocols were approved by the Algerian MESRS and DGRSDT Committee (D01N01UN250120230010) and are in accordance with the guidelines for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996) and the directives of the European Union regarding the handling of experimental animals (86/609/EEC).

Plant material

Samples of *Crotalaria vialattei* were collected in the flowering stage from Oued Tenia-Zerga Mogheul, Béchar Province, western Algeria (latitude: N 32°1'23.69", longitude: W 2°13'3.06"). The aerial parts of this plant were shade-dried under ambient conditions, crushed, and finely powdered using an electric grinder. Botanical authentication was performed by Dr Wafa Nouioua at Ferhat Abbas University, Setif 1, Faculty of Natural and Life Sciences, Department of Plant Ecology and Biology, Laboratory of Phytotherapy Applied to Chronic Diseases, Setif, Algeria, based on established prior work in the field [10]. A voucher specimen has been deposited in the Herbarium of the Faculty, under the order N°FSNV25/2025.

Preparation of the extract

The air-dried and finely ground aerial parts (1,200 g) of *C. vialattei* were macerated at ambient temperature

for 48 h using a hydromethanolic extraction system (methanol: water, 70:30, v/v). Following filtration, the combined extract was concentrated under reduced pressure at a maximum temperature of 35°C, yielding approximately 500 mL of concentrated solution. This residue was subsequently diluted with 420 mL of distilled water under continuous magnetic stirring and stored for one night at 4°C to facilitate the precipitation of chlorophylls. After filtration, the aqueous phase was progressively partitioned with solvents of rising polarity, namely petroleum ether, chloroform, ethyl acetate, and *n*-butanol. Each organic layer was treated with anhydrous sodium sulfate (Na_2SO_4), filtered, and evaporated under reduced pressure (≥35°C) to obtain the respective dried extracts: petroleum ether (5.41 g), chloroform (1.62 g), ethyl acetate (6.63 g), and *n*-butanol (22.47 g). The resulting fractions were stored at -20°C pending further analysis.

Phytochemical analysis

Total phenolic content

The Folin-Ciocalteu technique was employed with minor adjustments to assess the total phenolic content [11]. Briefly, a mixture of 100 µL of Folin-Ciocalteu and 20 µL of BCV was added to 75 µL of $NaCO_3$ (7.5%). The mixture was left in the dark for 2 h at ambient temperature, after which absorbance was measured at 765 nm. The phenolic concentration was calculated using a gallic acid calibration curve (y =1.8372x+0.1424, R^2 = 0.9909) within a linear range of 25-200 µg/mL**.** Results were expressed as micrograms of gallic acid equivalent per mg of the extract (µg GAE/mg).

Total flavonoid content

The total flavonoid content was assessed by the interaction of the extracts with sodium carbonate (Al^{+3}) [12]. Briefly, a mixture of 50 µL of BCV, 130 µL of methanol, 10 µL of CHCOOK solution (9.8 g/100mL distilled water), and 10 µL of (Al $(NO_3)_3$, $9H_2O$) solution (10%) was homogenized. After reacting for 40 min at 20-25°C, the optical density of the mixture was recorded at 415 nm. The flavonoid concentration was calculated using a quercetin calibration curve (y= 0.0048x, R^2= 0.997) within a linear range of 6.25-400 µg/mL**.** The data were expressed as micrograms of quercetin equivalent per mg of the extract (µg QE/mg).

LC-ESI-MS/MS analysis

The phenolic compounds in BCV were qualitatively and quantitatively determined using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) with an Agilent Technologies 1260 Infinity II system coupled to a 6460 Triple Quadrupole mass spectrometer, equipped with a Poroshell 120 SB-C18 column (3.0 × 100 mm, I.D., 2.7 µm). For LC-MS/MS analyses, 50 mg of the extract was placed into a 2-mL Eppendorf tube and then dissolved in 1 mL of methanol, followed by the addition of 1 mL of hexane. The mixture obtained was extracted using hexane. Subsequently, the final mixture was centrifuged at 7,200×g for 10 min. A 100-µL aliquot of the methanolic phase was diluted in 900 µL of a water/methanol mixture (5/5: v/v water/methanol). The final sample was filtered and transferred into vials. The mobile phase consisted of water containing 0.1% formic acid and 5.0 mM ammonium formate (A), and methanol containing 0.1% formic acid and 5.0 mM ammonium formate (B). The gradient program was modified at 25% for 1-3 min, 50% for 4-12 min, 90% for 13-21 min, and 3% for 22-25 min for the B mobile phase. The capillary voltage was 4,000 V, nebulizing gas (N_2) flow was 11 L/min, pressure was 15 psi, and gas temperature was 300°C [13]. The analysis was conducted using LC-MS/MS under the following conditions: injection volume of 5.12 µL, flow rate of 0.400 mL/min, method duration of 30 min, and column temperature maintained at 40°C [14].

***In vitro* antioxidant activity**

DPPH free-radical scavenging

The DPPH radical scavenging activity of BCV was evaluated using a spectrophotometric method [15]. Forty µL of the sample solution, as well as the standard BHT at varying concentrations, was mixed with 160 µL of DPPH solution (0.1 mM in methanol). The mixtures were incubated in the dark at room temperature for 30 min, after which the absorbance was measured at 517 nm. The results were expressed as IC_{50} (µg/mL) values.

Reducing power assay

The reducing power of the extract was assessed using the method described by [16]. A mixture of 10 µL of the sample, 40 µL of phosphate buffer (0.2 M, pH 6.6),

and 50 µL of 1% potassium ferricyanide was incubated at 50°C for 20 min. Then, 50 µL of 10% trichloroacetic acid, 40 µL of distilled water, and 10 µL of 0.1% $FeCl_3$ were added. Absorbance was recorded at 700 nm. Results were expressed as $A_{0.5}$ values.

Phenanthroline assay

The phenanthroline chelating activity of BCV was assessed spectrophotometrically [17]. Ten µL of the sample was mixed with 50 µL of $FeCl_3$ solution (0.2%), 30 µL of o-phenanthroline (0.5%), and 110 µL of methanol. The mixture was incubated at 30°C for 20 min, and absorbance was measured at 510 nm. Butylated hydroxytoluene (BHT) served as a reference compound, and results were expressed as $A_{0.5}$ values.

ABTS radical scavenging assay

The $ABTS^{\bullet+}$ radical scavenging activity was evaluated according to [18]. In a 96-well microplate, 40 µL of a methanolic sample solution (at various concentrations) was mixed with 160 µL of the $ABTS^{\bullet+}$ solution. After incubation at room temperature, absorbance was measured at 734 nm. Each measurement was performed 3 times. BHT was applied as a reference antioxidant.

***In vivo* hepatoprotective effect**

Animal care

Healthy male *Wistar albino* rats, weighing between 180 g and 220 g, were provided by the animal house of the Faculty of Natural and Life Sciences, University Constantine 1 Frères Mentouri. The animals were kept in uniform plastic enclosures fitted with stainless steel lids, with each cage accommodating between 3 and 6 rats. Animals were allowed unrestricted access to standard laboratory chow and water. Before the initiation of the experimental protocols, all animals underwent a 2-week acclimatization period under controlled environmental settings, including a constant ambient temperature of 22±2°C and a 12:12-h light/dark photoperiod, to ensure stabilization of their circadian rhythms. All experimental protocols were conducted in strict adherence to ethical guidelines and institutional recommendations for the care and treatment of laboratory animals.

Experimental design

Hepatotoxicity was induced using a fixed-dose formulation of an antitubercular drug containing 3 active substances: rifampin 150 mg/kg, isoniazid 75 mg/kg, and pyrazinamide 400 mg/kg. This medication is in the form of tablets meeting USP standards (manufactured by Deutsche Labs, India), and it is commonly referred to by the abbreviation RHZ. Based on previous laboratory observations regarding the effects of plant extracts on xenobiotic-induced hepatotoxicity [19], 2 doses of BCV (100 and 150 mg/kg) were selected. All treatments were solubilized in distilled water and administered orally to rats divided into 6 groups (n=6 per group) as follows: Group 1 (Control) received distilled water only; Group 2 (RHZ), serving as the hepatotoxic control, received RHZ (rifampin 150 mg/kg, isoniazid 75 mg/kg and pyrazinamide 400 mg/kg); Groups 3 and 4 (BCV 100 mg/kg; BCV 150 mg/kg) received BCV only at 100 mg/kg and 150 mg/kg, respectively; while Groups 5 and 6 (BCV 100 mg/kg + RHZ; BCV 150 mg/kg +RHZ) were administered BCV (100 or 150 mg/kg) 1 h prior to initiation of RHZ treatment.

After 21 days of treatment, the rats were anesthetized and sacrificed by dissection. Blood samples were collected from the hepatic portal vein into heparinized tubes for the analysis of biochemical parameters. The livers were carefully excised for histopathological examination and the evaluation of the pro-/antioxidant status.

Biochemical parameters

The serum was separated by centrifugation at 400×g for 10 min and subsequently used for the assessment of hepatic biochemical markers. ALT, AST, ALP, triglycerides, and cholesterol were measured using commercial assay kits (Spinreact, Spain) with a semi-autoanalyzer (Transasia, Model ERBA, CHEM 5V2). Enzymatic activities are expressed in international units per liter (IU/L), whereas triglyceride (TG) and cholesterol concentrations are expressed in grams per liter (g/L)

Liver tissue oxidant/antioxidant status

Hepatic tissues intended for the evaluation of *in vivo* antioxidant activity were homogenized at a 20% (w/v)

concentration in a cold 1.15% KCl solution, and then centrifuged at 400×g for 15 min at 4°C. The resulting supernatants were used for the determination of antioxidant enzyme activities and malondialdehyde (MDA) levels.

Lipid peroxidation determination

Lipid peroxidation levels were determined in the supernatants of liver tissue homogenates using the thiobarbituric acid reactive substances (TBARS) assay, based on a colorimetric method [20]. The reaction mixture consisted of 0.5 mL of 20% (w/v) liver homogenate, 3 mL of 1% phosphoric acid, and 1 mL of 0.67% thiobarbituric acid (TBA). Following incubation in a boiling water bath for 45 min, 4 mL of *n*-butanol was added to the reaction mixture. The samples were then centrifuged, and the absorbance was measured at 532 nm.

Intracellular reduced glutathione levels

Hepatic reduced glutathione (GSH) content was determined using Ellman's reagent [21]. This colorimetric assay is based on the reaction of 5.5'-dithiobis (2-nitrobenzoic acid) (DTNB) with sulfhydryl groups, resulting in the formation of a yellow-colored chromophore with a maximal absorbance at 412 nm, measured against a reagent blank.

Glutathione peroxidase (GPx) activity

In this assay, glutathione peroxidase (GPx) catalyzes the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH) [22]. Briefly, 0.2 mL of hepatic tissue homogenate was mixed with 0.4 mL of 0.1 mM GSH and 0.2 mL of Tris-buffered saline (TBS: 50 mM Tris-HCl, 150 mM NaCl, pH 7.4), and incubated at 25°C. After 5 min, 0.2 mL of 1.3 mM H_2O_2 was added, and the mixture was further incubated for 10 min. The reaction was terminated by the addition of 1 mL of 1% trichloroacetic acid (TCA), followed by incubation in an ice bath (0-5°C) for 30 min. Samples were then centrifuged at 400 × g for 10 min. Subsequently, 0.48 mL of the supernatant was mixed with 2.2 mL of TBS and 0.32 mL of 1 mM DTNB. After 5 min of incubation, absorbance was measured at 412 nm.

Histopathological study

Liver samples were fixed in 10% neutral buffered formalin for 48 h, dehydrated through a graded ethanol series, and embedded in paraffin wax. Sections (3-5 µm thick) were prepared using a microtome, deparaffinized in xylene, and stained with Harris hematoxylin and eosin (H&E). Histopathological changes were examined and photographed using a light photomicroscope (LEICA DM750, Germany).

Statistical analysis

Data are expressed as the mean±standard deviation (SD). For *in vitro* assays, all experiments were performed in triplicate (n=3). For *in vivo* experiments, each experimental group consisted of 6 animals (biological replicates, n = 6). For each rat, biochemical and oxidative stress parameters were measured in triplicate (technical replicates), and the mean value was used for statistical analysis. Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. All statistical evaluations were performed using GraphPad Prism software version 8.4.3 (San Diego, CA, USA). Results with P-values < 0.05 were considered statistically significant.

RESULTS

Total phenolic and flavonoid content

The phytochemical composition of the BCV extract, as shown in Table 1, was assessed through quantitative spectrophotometric analyses. The data show substantial total phenolic content (TPC) and total flavonoid content (TFC), with values of 153.81±0.7 µg GAE/mg and 86.44±0.1 µg QE/mg of dry extract, respectively.

LC-MS/MS analysis

Chromatographic fingerprints of BCV were characterized using liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. The LC-MS/MS chromatogram of the analyzed extract is provided as Supplementary Fig. S1. Identified compounds, their quantitative data, retention time (RT), coefficient of

Table 1. Total phenolic and flavonoid contents and antioxidant potential of BCV. Total phenolic content (TPC) and total flavonoid content (TFC) values of the extract, as well as IC_{50} and $A_{0.5}$ values of BCV, compared with butylated hydroxytoluene (BHT) used as a reference antioxidant

	Phenolic content		Reducing power $A_{0.5}$ (µg/mL±SD)		Radical scavenging activity IC_{50} (µg/mL±SD)	
Test	**TPC (µg GAE/mg)**	**TFC (µg QE/mg)**	**FRAP**	**Phenanthroline**	**DPPH**	**ABTS**
BCV	153.81±0.7	86.44±0.11	336±0	33.87±1.27	188.67±3.46	114.81±1.28
BHT	/	/	>50	0.93±0.07	22.32±1.19	12.99±0.41

TPC – total phenolic content; GAE – gallic acid equivalent; QE – quercetin equivalent; TFC – total flavonoid content; DPPH – 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay; FRAP – ferric reducing antioxidant power assay; ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay; BHT – butylated hydroxytoluene; BCV – *n*-butanol extract of *Crotalaria vialattei*; SD – standard deviation; IC_{50} – sample concentration at which 50% of the free radicals activity was inhibited; $A_{0.5}$ – concentration corresponding to an absorbance of 0.5. Values are expressed as mean±SD of 3 parallel measurements.

Table 2. LC-MS/MS of the *n*-butanol extract of *C. vialattei* (mg/g extract).

Compound	Retention time (min.)	R^2	Concentration (mg/g)	LOQ (ug/L)	LOD (ug/L)	Range (ug/L)
Vanillic acid	**9.85**	0.996	0.016807	219.042	664.37	664-2000
Hydroxybenzaldehyde	**10.00**	0.999	0.02009	4.974	15.07	15-2000
Syringic acid	**10.14**	0.999	0.036859	358.500	1086.26	500-5000
Vanillin	**10.64**	0.995	0.007913	14.588	44.21	20-1000
O-coumaric acid	**11.23**	0.999	0.004036	3.535	10.71	10-500
Trans-ferulic acid	**11.45**	0.995	0.012731	1.761	5.34	5-1500
Polydatin	**11.57**	0.997	0.075109	435.00	1318.18	1000-5000
Resveratrol	**11.62**	0.991	0.15279	4.581	13.88	10-10000
Rutin	**11.83**	0.996	0.011538	59.560	180.48	180-4000
Isoquercitrin	**12.04**	0.998	0.041848	9.938	30.12	10-5000
Hesperidin	**12.10**	0.996	0.0034975	4.140	12.54	12.5-500
Biochanin A	**13.05**	0.996	0.13069	0.148	0.45	0.45-10000
Morin	**13.56**	0.998	0.02129	0.125	0.38	0.35-2000
Naringenin	**13.57**	0.996	0.0017743	1.369	4.15	4-500
Trans-cinnamic acid	**13.66**	0.999	0.11028	11.185	33.88	30-10000
Chrysin	**15.13**	0.999	0.037775	0.074	0.22	0.2-5000
Diosgenin	**21.19**	0.998	0.0014827	2.933	10.89	10-250

RT – retention time; R^2 – coefficient of determination; LOQ – limit of quantification; LOD – limit of detection. ND – not determined. LOD and LOQ were calculated according to the ICH approach using the calibration slope (S) and the standard deviation of the response (σ) as LOD = 3.3σ/S and LOQ = 10σ/S. The lower limit of the linear range was set equal to the LOQ for each compound

determination (R^2), limit of quantification (LOQ), and limit of detection (LOD) are given in Table 2.

A total of 17 phenolic compounds were identified, with resveratrol, biochanin A, and trans-cinnamic acid as the dominant constituents (0.15279, 0.13069, and 0.11028 mg/g, respectively). These comprised five phenolic acids (vanillic, syringic, trans-ferulic, *o*-coumaric, and trans-cinnamic acids); seven flavonoids, with biochanin A as the predominant compound (0.13069 mg/g), along with rutin, hesperidin, naringenin, morin, isoquercitrin, and chrysin; two stilbenes (resveratrol and polydatin); two aromatic phenolic aldehydes (vanillin and hydroxybenzaldehyde); and one steroidal compound, diosgenin, associated with saponins.

Antioxidant activity

The results showed that the extract exhibited the highest activity in the phenanthroline assay, with an $A_{0.5}$ value of 33.87±1.27 µg/mL, followed by notable radical scavenging activity in the ABTS and DPPH assays, with IC_{50} values of 114.81±1.28 µg/mL and 188.67±3.46 µg/mL, respectively. Additionally, the plant extract exhibited moderate reducing power in the FRAP assay with an $A_{0.5}$ value of 336±0, as illustrated in Table 1. Taken together, the results of all *in vitro* assays confirm that this plant possesses effective antioxidant activity, supporting its potential use as a natural antioxidant agent.

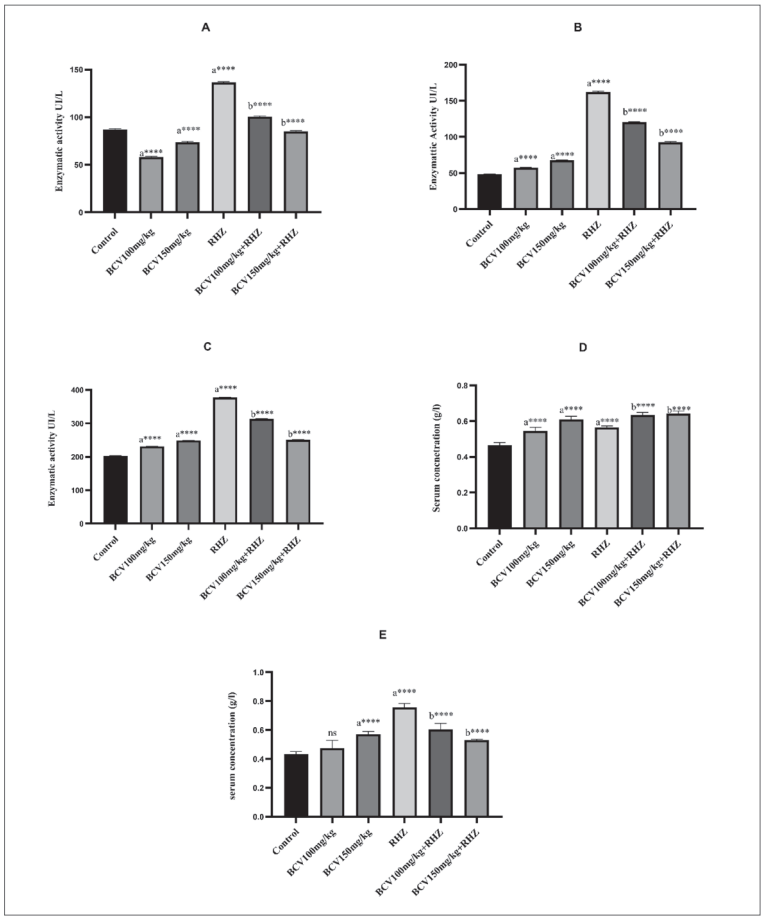

Fig. 1. Effect of RHZ and BCV on serum biomarkers of hepatic injury and on lipidic profile *in vivo*. **A** –aspartate transaminase (AST); **B** – alanine transaminase (ALT); and **C** – alkaline phosphatase (ALP); **D** – cholesterol levels; **E** – triglyceride levels (TG); CV – *Crotalaria vialattei*: BCV – *n*-butanol extract of *Crotalaria vialattei*. Data represent mean±SD (n=6 biological replicates), with each parameter measured in technical triplicate and differences between the different groups were estimated using one-way ANOVA followed by Tukey's multiple comparison test. **a:** significantly different compared to the control group; **b**: significantly different compared to the RHZ-treated group (****P<0.0001; ns – not significant).

Hepatoprotective effect of BCV extract

Serum biomarkers of hepatic injury

The effects of RHZ and BCV on serum markers of liver injury are shown in Fig. 1A-C. Administration of RHZ resulted in a significant increase (P<0.0001) in AST, ALT, and ALP levels compared to the control group, with values rising from 87.17±0.75, 48.17±0.75. and 202±0.89, respectively, to 136.88±0.7, 162.2±1.16, and 377.7 ±0.81. Pretreatment with two doses of BCV extract (100 and 150 mg/kg) significantly reduced the elevated serum levels of AST, ALT, and ALP (P < 0.0001). In the BCV + RHZ groups, AST levels decreased to 100.8±0.75 and 85.17±0.75 at doses of 100 and 150 mg/kg, respectively, while ALT levels declined to 120.5±0.54 and 92.5±1.04 (Fig. 1A, B). Similarly, serum ALP levels were significantly reduced to 313.3±0.81 and 250.8±0.75, respectively (Fig. 1C). Administration of BCV alone at doses of 100 and 150 mg/kg did not increase serum AST levels; instead, a significant decrease was observed at both doses (58.17±0.75 and 73.5±1.04). In contrast, BCV alone induced a significant increase (P<0.0001) in serum ALT and ALP levels at both doses compared with the control group; however, these increases were lower in magnitude than those observed in the RHZ-treated group (Fig. 1B, C).

Effects on serum lipid profile

As illustrated in Fig. 1D, E, both RHZ and BCV treatments induced changes in the lipid profile. RHZ administration resulted in a highly significant increase in serum cholesterol and triglyceride (TG) levels compared with the control group (cholesterol: 0.57±0.008 g/L; TG: 0.75±0.02 g/L; P<0.0001). BCV pretreatment significantly attenuated the RHZ-induced increase in TG levels (P<0.0001), whereas no significant effect was observed on cholesterol levels (Fig. 1D, E).

Administration of BCV alone at 100 mg/kg resulted in a non-significant increase in TG, whereas administration at 150 mg/kg induced a highly significant elevation (P<0.0001) compared to the control. For both BCV doses, total cholesterol levels were significantly higher than in the control group (P<0.0001).

Effects of BCV extract on lipid peroxidation, cell glutathione, and liver antioxidant enzymes

The impact of RHZ and BCV treatments on hepatic redox markers is presented in Fig. 2. RHZ administration induced a significant elevation in MDA levels, which rose from 0.14± 0.007 to 0.2±0.01 nmol/mg tissue (P<0.0001; Fig. 2A). Pretreatment with BCV at

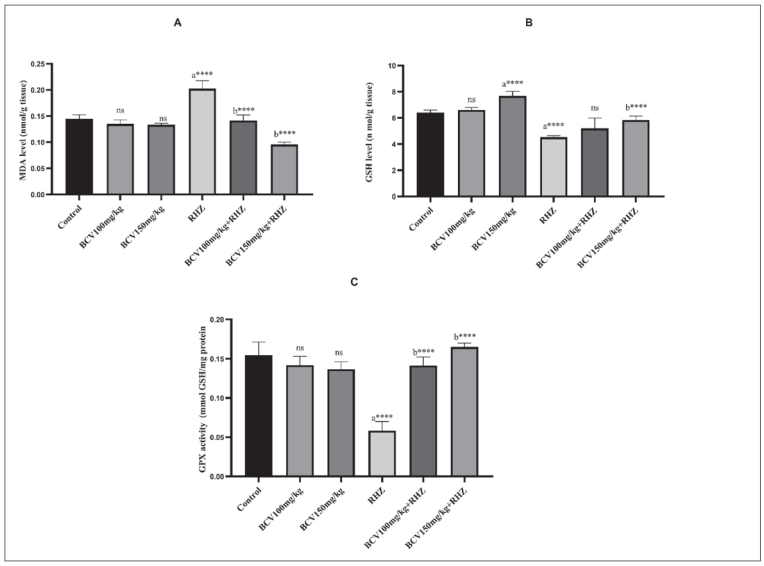

Fig. 2. Effect of RHZ and the BCV extract on the pro/antioxidant parameters levels in hepatic tissues. **A** – MDA levels; **B** – GSH levels; **C** – GPx activity; CV – *Crotalaria vialattei*; BCV – *n*-butanol extract of *Crotalaria vialattei*. Data are the mean±SD (n=6 biological replicates), with each parameter measured in technical triplicate and differences between the different groups were estimated using one-way ANOVA followed by Tukey's multiple comparison test. **A** – significantly different compared to the control group; **b** – significantly different compared to the RHZ-treated group (****P<0.0001; ns – not significant).

100 and 150 mg/kg significantly reduced these concentrations to 0.141±0.01 and 0.09±0.005 nmol/mg protein, respectively (P<0.0001). Groups treated with BCV alone showed no induction of lipid peroxidation, maintaining levels similar to the control (Fig. 2A). Regarding the antioxidant defense system, RHZ treatment led to a significant depletion of GSH levels (4.52 ±0.12) compared to the control group (7.67±0.34, P< 0.0001; Fig. 2B). Pretreatment with BCV at 150 mg/kg significantly attenuated this reduction (5.84±0.3, P< 0.0001), while the 100 mg/kg dose showed a non-significant increase. In rats receiving BCV alone at 150 mg/kg, a significant increase in GSH levels was observed compared to the control (Fig. 2B). Similarly, a marked reduction in GPx activity was observed in the RHZ-treated group (0.05±0.01) compared to the control (0.15±0.01, P<0.0001; Fig. 2C). Pretreatment with BCV at 100 and 150 mg/kg restored the enzymatic activity to 0.14±0.01 and 0.16±0.004, respectively (P<0.0001). Administration of BCV alone did not significantly alter the baseline activity of this enzyme (Fig. 2C). The results indicate a dose-dependent modulation of these oxidative markers by the extract.

Histopathological examination of liver tissue

Histopathological examination of liver tissues from the control group revealed normal architecture, with hepatocytes arranged in organized cords delineated by distinct sinusoidal capillaries (S) and hexagonal lobules centered on a preserved central vein (CV) (Fig. 3A). In contrast, the RHZ-treated group exhibited parenchymal alterations, characterized by hepatocyte ballooning with nuclear hyperchromasia (NC), suggesting a pre-necrotic state (Fig. 3D). Specific areas showed nuclear loss (NL), portal inflammatory cell infiltration (P) (Fig. 3B), and vascular congestion (Fig. 3C). Rats pretreated with BCV exhibited a largely preserved hepatic architecture, although localized injury and portal congestion (C) were still evident (Fig. 3E). Notably, administration of BCV (100 and 150 mg/kg) before RHZ preserved the hepatic parenchyma, with only limited foci of cellular ballooning observed (Fig. 3F, G).

DISCUSSION

Based on the available literature, the present study contributes to the characterization of *Crotalaria vialattei* and supports its pharmacological relevance as an underexplored species and a potential source of therapeutic agents for the management of drug-induced liver disorders.

The phytochemical profile of BCV revealed a rich composition of polyphenols and flavonoids. Although other *Crotalaria* species have been reported to contain high phenolic contents [7], total phenolic and flavonoid levels alone do not fully reflect the unique phenolic fingerprint of a given species [23]. Accordingly, LC-MS/MS analysis identified 17 phenolic compounds in BCV. Several of these compounds were also reported in *C. retusa*, including myricetin, quercetin, rutin, luteolin, apigenin, p-hydroxybenzoic acid, protocatechuic acid, and p-coumaric acid [24]. The use of an intermediate-polarity solvent such as *n*-butanol effectively favored the extraction of these flavonoids and phenolic acids [25].

Given the substantial phenolic content of BCV, its antioxidant potential was evaluated using standardized

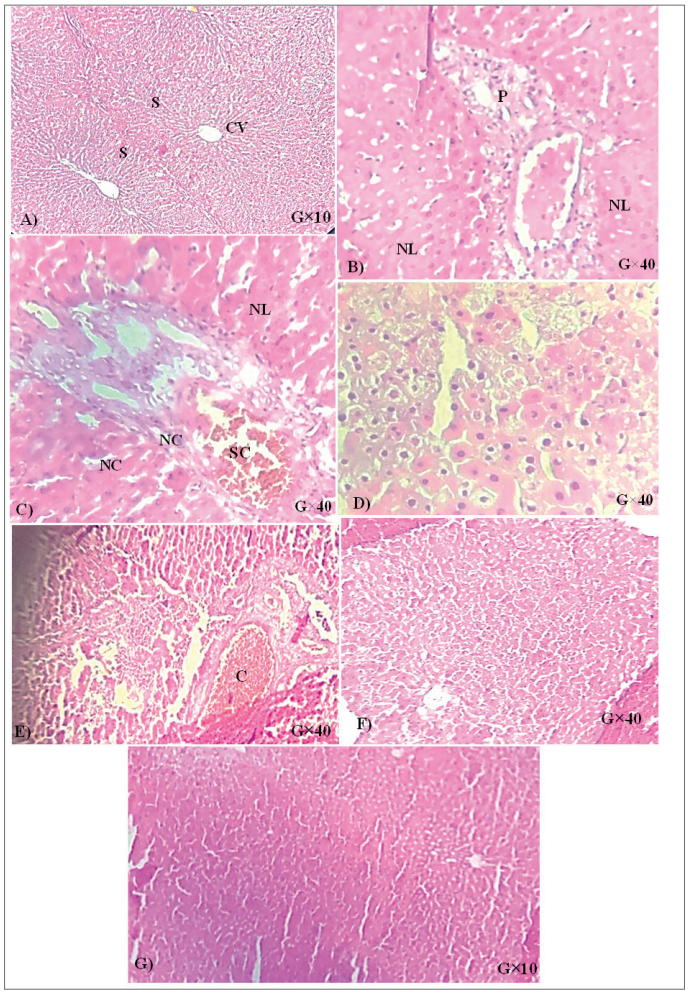

Fig. 3. Photomicrographs of H&E-stained rat liver sections. **(A)** Control group: Normal hepatic architecture featuring hepatocytes arranged in organized cords, distinct sinusoidal capillaries (S), and intact central veins (CV). **(B, C, D)** RHZ-treated group: Extensive parenchymal damage characterized by inflammatory cell infiltration (P), sinusoidal congestion (SC), hepatocyte ballooning, nuclear hyperchromasia (NC), and loss of nuclei (NL). **(E)** BCV-only groups (BCV100mg/kg and BCV150 mg/kg): Preserved hepatic parenchyma, showing only minor focal congestion (C) within the portal area. **(F, G)** RHZ + BCV treated groups (RHZ+BCV100mg/kg and RHZ+BCV150mg/kg): Significant restoration of hepatic architecture with localized and diminished hepatocellular ballooning compared to the RHZ group.

in vitro assays. The extract exhibited measurable antioxidant activity, although its potency was lower than that of the synthetic reference antioxidant. Variability among assays was observed, with lower activity in the FRAP assay and higher activity in the phenanthroline assay, as also noted for other *Crotalaria* species [9,26,27]. The antioxidant activity observed in BCV is closely associated with its high phenolic content, which is a recognized contributor to antioxidant capacity [28].

LC-MS/MS analysis consistently revealed several phenolic biomolecules in BCV, including flavonoids such as rutin and isoquercitrin, as well as vanillin and polydatin, which have been widely reported for their antioxidant and reducing capacity [29-32].

Antitubercular therapy with INH, RIF, and PZA is well known to induce hepatotoxicity, mainly through excessive generation of reactive oxygen species leading to liver injury [33]. In this context, the present study evaluated the hepatoprotective potential of BCV *in vivo* against RHZ-induced liver toxicity. RHZ-treated rats exhibited a significant increase in MDA, together with marked depletion in GSH levels and GPx activity, indicating enhanced lipid peroxidation and impairment of the endogenous antioxidant defense system [34,35]. Literature data indicate that the hepatotoxicity of these drugs increases synergistically when administered in combination. [36,37,3]. Oxidative stress represents a key event in RHZ-induced injury. Co-administration of BCV significantly attenuated this injury, as evidenced by reduced lipid peroxidation and restored antioxidant status. This protective effect is associated with the antioxidant-related properties of the phenolic constituents identified in the extract, including flavonoids such as rutin and naringenin, which have been widely reported for their radical scavenging capacities [38,39].

These alterations in RHZ-treated rats were further supported by biochemical markers. Administration of RHZ resulted in marked elevations in ALT, AST, and ALP, as well as increased TG and cholesterol levels, indicating drug-induced hepatotoxicity and disruption of lipid metabolism [36]. BCV co-administration significantly mitigated these biochemical changes, suggesting a protective effect that can be associated with polyphenolic constituents such as vanillic acid and polydatin, which are known to reduce hepatic enzyme levels, lipid peroxidation, and lipid accumulation in experimental models. [30,40].

Histopathological examination corroborated the biochemical findings. RHZ-treated rats showed severe hepatic alterations, whereas BCV treatment preserved normal hepatic architecture. Comparable efficacy has

been demonstrated for *C. longipes* against CCl_4-induced changes stemming from the combined action of several phytochemicals, especially flavonoids, which exert antioxidant activity and upregulate the expression of antiapoptotic factors [41,39].

Importantly, administration of BCV alone did not induce significant oxidative stress or histopathological changes in hepatic tissue. Although mild increases in ALP, ALT, cholesterol, and TG levels were observed, they suggest that the extract may modulate specific hepatic metabolic pathways. Given the scope of the study, these biochemical fluctuations should be interpreted with scientific caution. To definitively establish the safety profile of *C. vialattei,* further sub-chronic and chronic toxicity models are necessary.

The present study demonstrates that the *n*-butanol extract of *C. vialattei* exerts antioxidant-associated hepatoprotective effects against RHZ-induced liver injury, primarily through preservation of oxidative balance and hepatic structural integrity. Further studies are warranted to elucidate the precise mechanisms underlying these effects and to assess the long-term metabolic impact of this extract.

CONCLUSIONS

The present study demonstrates that the *n*-butanol extract of *Crotalaria vialattei* (BCV) exhibits antioxidant-associated hepatoprotective effects against RHZ-induced liver injury. The identification of 17 polyphenolic compounds highlights the richness of its phenolic profile and supports its measured antioxidant activity observed *in vitro* across multiple assays. *In vivo*, BCV pretreatment attenuated RHZ-induced hepatic alterations, as reflected by improvements in biochemical parameters, lipid profile, oxidative stress markers, and liver histoarchitecture. These findings suggest that the hepatoprotective effect of BCV is closely associated with its antioxidant properties and its ability to preserve hepatic redox balance and structural integrity. Overall, *C. vialattei* may represent a potential natural source of bioactive compounds with hepatoprotective relevance in the context of antituberculosis drug-induced toxicity. Nevertheless, further studies, including extended clinical trials, are required to better understand the underlying mechanisms and to assess the safety, efficacy, and possible drug-plant interactions.

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Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: The data supporting this article are available in the online dataset: https://www.serbiosoc.org.rs/NewUploads/Uploads/Zouioueche%20et%20al_Dataset.xlsx

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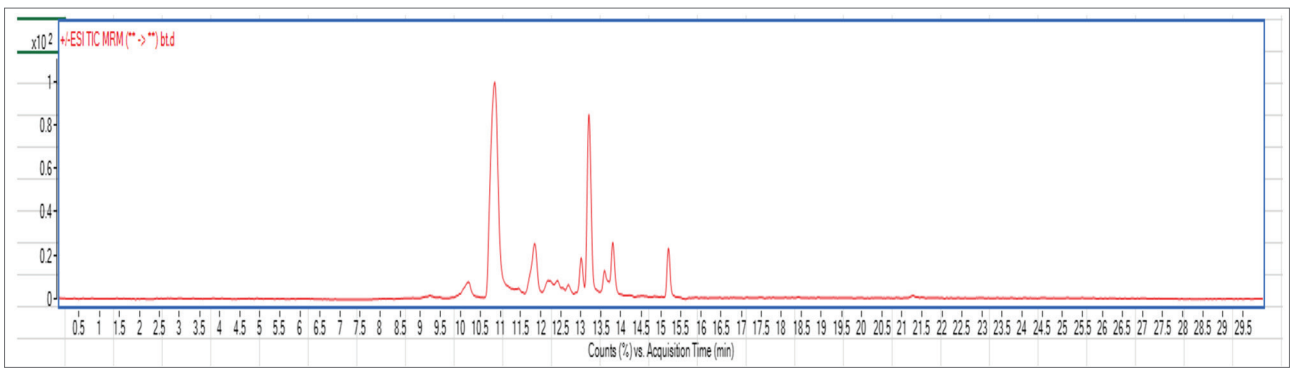
SUPPLEMENTARY MATERIAL



Supplementary Fig. S1. LC-MS/MS chromtogram of the *n*-butanol extract of *C. vialattei.* Illustrating the separation of multiple polyphenolic compounds in the analyzed sample, with distinct peaks corresponding to individual polyphenols observed at different retention times.