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Grape seed and skin extract protects against bleomycin-induced oxidative stress in rat lung

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ABSTRACT

Introduction: Lung fibrosis is a common side effect of the chemotherapeutic agent bleomycin and current evidence suggests that reactive oxygen species play a key role in the development of lung injury. We examined whether grape seed and skin extract (GSSE), a polyphenolic mixture exhibiting antioxidant properties, is able to protect against bleomycin-induced lung oxidative stress and injury.
Methods: Rats were pre-treated during three weeks either with vehicle (ethanol 10% control) or GSSE (4 g/kg), then administered with a single high dose bleomycin (15 mg/kg) at the 7th day.
Results: Bleomycin increased lung lipoperoxidation, carbonylation and decreased antioxidant enzyme activities as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Bleomycin also induced copper depletion from the lung and iron accumulation within the lung, but had no effect on either zinc nor manganese. Correlatively bleomycin decreased the copper associated enzyme tyrosinase, increased the zinc dependent lactate dehydrogenase (LDH) and did not affect the manganese dependent glutamine synthetase. GSSE efficiently counteracted almost all bleomycin-induced oxidative stress, biochemical and morphological changes of lung tissue.
Conclusion: Data suggest that GSSE exerts potent antioxidant properties that could find potential application in the protection against bleomycin-induced lung fibrosis.
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1. Introduction

Bleomycin (Bleo) is an effective chemotherapeutic agent against a broad spectrum of human cancers such as esophageal and squamous cell carcinomas [43]. Treatment with Bleo is very much limited because of the development of dose and time-dependent pneumonitis that can progress to interstitial pulmonary fibrosis [7]. Interstitial pulmonary fibrosis is characterized by an altered cellular composition of the alveolar region with excessive deposition of collagen. Additionally, lung inflammation is a major underlying component of a wide variety of pulmonary fibro proliferative disorders and reactive oxygen species (ROS) [23].

The mechanisms of Bleo-induced pulmonary injury and fibrosis are not completely understood. Bleo is able to generate ROS upon binding to DNA and iron and the interaction of Bleo with DNA initiates the inflammatory and fibro proliferative change via a concerted action of various cytokines leading to collagen accumulation. Bleo also promotes the depletion of endogenous antioxidant defenses thus exacerbating oxidative mediated tissue injury [4]. Strategies aiming at the reduction of oxidative stress include the use of superoxide dismutase [16], glutathione [35], dimethylurea [45], metalloporphyrin [33] and the prominent role of ROS has led to the development of new antioxidants.

Grape seed and skin extract (GSSE) has many beneficial health effects including cardio-protective, reno-protective, hepato-protective and neuroprotective [30]. GSSE is also protective against the toxic side effects linked to the use of antineoplastic drugs as doxorubicin [36], cisplatin [48] and bleomycin [2]. GSSE is a complex mixture of bioactive components including proanthocyanidins, flavonoids and stilbenes [22]. Flavonoids are highly concentrated in grape seeds and mainly composed of monomeric catechins and flavonols as quercetin [6]. Non-flavonoids, highly present in grape skin contained stilbenes as resveratrol which is at the basis of the French Paradox [37]. Proanthocyanidins exert antineoplasic effects by cell cycle arrest and induction of apoptosis [20].

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Table 1
Phenolics levels in GSSE.

Phenolics	Seed	Skin
Total phenolics (mg/g extract)	67	51
Total Flavonoids (mg/g extract)	16	14
Non flavonoids (mg/g extract)	51	37
Condensed tannins (mg/g extract)	1.22	3.43
Total anthocyanins (μg/g extract)	0.997	0.962

Based on this background we aimed in the present study to evaluate the putative protective effect of GSSE in Bleo-induced lung oxidative stress injury in rat. Data showed GSSE as a potential preventive agent against the deleterious damages of Bleo into the lung.

2. Materials and methods

2.1. Chemicals

Thiobarbituric acid (TBA); 2,6,-di-*tert*-butyl-4-hydroxy-toluene (BHT); trichloroacetic acid (TCA); hydrogen peroxide (H_2O_2); bovine catalase; 4-(1-Hydroxy-2-methylamino-ethyl)-benzene-1,2-diol (epinephrine) and 2,4,dinitro-phenyl hydrazine (DNPH) were obtained from Sigma-Aldrich Co. (Germany); Bleomycin-sulfate was obtained from Sanofi-aventis (France).

2.2. Composition of grape seed and skin extract

GSSE was processed from a grape cultivar (Carignan) of *Vitis vinifera* from northern Tunisia. Seeds and skin were dried and grounded separately with an electric mincer (FP3121 Moulinex) until a fine powder was obtained. Powder mixture containing grape seed (50%) and skin (50%) was dissolved in 10% ethanol in the dark, vigorously vortexed for 15 min, centrifuged at 10,000*g* for 15 min at 4 °C for debris elimination and supernatant containing soluble polyphenols was ready to use.

Table 2
LC–MS/MS data of some phenolic compounds found in GSSE.

Compounds	*m/z* negative mode [MH]-	MS2 fragment	Relative abundance (%)	
			Seed	Skin
Catechin	289	245/108.8/122.8	2.27	0.36
Epicatechin	289	245/108.8/122.8	2.85	0.37
Procyanidin dimmer	577	289.3/407.4	0.47	ND
Procyanidintrimer	865	577	ND	ND
Quercetin	301	150.8/120.9	0.64	0.47
Resveratrol	227	184.6/143	0.14	ND
Rutin	609.19	300.1	1.51	0.5
Vanillin	151.14	135.7/108.1	10.67	7.75
Gallic acid	169	124.7/78.9	50.3	32.77
P-coumaric acid	163	119/93	ND	0.38
Rosmarinic acid	359.2	160.8/197.1	ND	0.75
2,5dihydroxybenzoic acid	152.7	108.7/90.7	30.58	51.96
Caffeic acid	179	135	ND	2.8
Chlorogenic acid	353	191	ND	0.34
Ferulic acid	193	134/89	0.55	1.46

Total phenolic content was determined by the Folin-Ciocalteau colorimetric method [40], flavonoids and condensed tannins according to Dewanto et al. [11] and Sun et al. [42] respectively. GSSE composition was established by HPLC–MS/MS analysis. Briefly liquid chromatography was performed using a Perkin Elmer system series 200 equipped with a binary micro-pump. The analyses were carried out on a C18 column (Zorbax Eclipse XDB-C18, 4.6 × 150 mm, particle size 5 μm). The mobile phase A was 0.1% formic acid in water and the mobile phase B was 0.1% formic acid in acetonitrile. Elution was performed at a flow rate of 1 mL min^{-1} and an injection volume of 20 μL. Tandem mass spectrometry (MS/MS) was carried out using a 3200 QTRAP mass spectrometer (Applied Biosystems/MDS Sciex Forster city USA) equipped with an electrospray ionization (ESI) interface. Data were acquired and processed with Analyst 1.5.1 software. The detector was set in the negative ion mode. The ion trap mass spectrometer was operating in the *m/z* 50–1700 mass range.

2.3. Animals and treatment

Twenty-four male Wistar rats (195–200 g) from Pasteur Institute (Tunis) were used in agreement with the ethic committee of Tunis University and with NIH guidelines [31]. They were provided with food and water ad libitum and maintained in animal house at fixed temperature of 22 ± 2 °C with a 12 h light–dark cycle. Rats were randomly divided into four groups of six animals each that were daily treated by intraperitoneal (ip) route.

- Group 1: control: rats receiving 10% ethanol for 21 days
- Group 2: GSSE: rats receiving 4 g/kg GSSE for 21 days
- Group 3: Bleo: rats receiving a single dose of Bleo (15 mg/kg) at day 7.
- Group 4: Bleo + GSSE: rats treated both with GSSE and a single dose of Bleo at day 7.

At the end of the treatment, rats were anesthetized with urethane (40 mg/mL), sacrificed, their blood collected into heparinized tubes and processed for plasma biomarkers determination. Lungs were isolated, homogenized in PBS buffer pH 7.4 with an ultrathurax T25 homogenizator, centrifuged (15 min at 10,000*g*, 4 °C) and the resulting supernatant was used for lung lipidemia and oxidative stress analyses.

2.4. Biochemical analyses

Lung lipids were extracted according to Folch et al. [15]. Triglyceride and total cholesterol were determined using commercially available kits from Biomaghreb Tunisia.

Malondialdehyde (MDA), a marker of lipid peroxidation was determined according to Draper and Hadley [12]. An aliquot of the homogenate was mixed with butylated hydroxy toluene/trichloroacetic acid (BHT/TCA) solution containing 1% (*w/v*) BHT dissolved in 20% TCA (*w/v*) and centrifuged at 4000*g* for 15 min at 4 °C. Supernatant was blended with 0.6 N HCl and 120 mmol L^{-1}

Table 3
Effect of bleomycin and GSSE on body weight, lung weight and index.

	C	GSSE	Bleo	Bleo + GSSE
Initial body weight(g)	195.600 ± 2.848	198.300 ± 5.890	199.000 ± 4.312	198.500 ± 4.800
Final body weight(g)	211.000 ± 6.450	210.750 ± 6.250	184.000 ± 4.500	202.750 ± 1.108
Lung weight(g)	0.211 ± 0.079	0.210 ± 0.021	0.184 ± 0.042	0.202 ± 0.055
Lung index	0.0045 ± 0.0002	0.0045 ± 0.0001	0.0077 ± 0.0001	0.0066 ± 0.0002

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