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Acacia tortilis ssp. *raddiana*: Analgesic and anti-inflammatory activities and early therapeutic effects in pulmonary fibrosis

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ABSTRACT

Introduction: *Acacia tortilis* ssp. *raddiana* is rich in bioactive phytochemicals, particularly phenolic constituents, exhibiting antioxidant and anti-inflammatory activities. Therefore, this study investigated the phytochemical composition and analgesic, anti-inflammatory, and anti-fibrotic effects of *Acacia raddiana*.

Methods: The antinociceptive and anti-inflammatory effects of the hydroethanolic leaf extract (0.5–1 g/kg) were evaluated in Swiss albino mice using the models of thermal nociception, chemical nociception, and carrageenan-induced paw inflammation. The early therapeutic effects of the leaf extract and gum solution were assessed in an experimental pulmonary fibrosis model induced by bleomycin. The phytochemical composition was investigated using standard qualitative and quantitative methods based on precipitation and colorimetric reactions. Moreover, the leaf extract was analyzed by high-performance liquid chromatography (HPLC) to identify phenolic compounds.

Results: Phytochemical screening revealed flavonoids, saponins, tannins, anthraquinones, coumarins, steroids, and triterpenes in the leaf extract, and saponins, terpenes, sterols, and coumarins in the gum. The polysaccharide content of the gum was estimated at 58.8% (w/w). HPLC analysis showed that rutin was the major constituent of the leaf extract (24%). Pretreatment with the extract (0.5–1 g/kg, p.o.)significantly reduced acetic acid–evoked writhing, carrageenan-mediated paw swelling, and increased reaction latency in the hot plate test ($P<0.01$). Both the extract and gum attenuated weight loss, oxidative damage, pulmonary collagen accumulation, and inflammatory alterations associated with bleomycin-mediated lung injury.

Conclusion: These results may support the therapeutic potential of *A. tortilis* ssp. *raddiana* in pain, inflammation, and pulmonary fibrosis.

Implication for health policy/practice/research/medical education:
The findings of this study support the potential of *Acacia raddiana* in evidence-based traditional medicine, with possible applications in the treatment of pain, inflammation, and fibrosis, pending clinical validation. They also highlight the need for further research to isolate active compounds, clarify mechanisms of action, and conduct clinical trials, as well as the importance of integrating phytotherapy into medical education.

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a disorder of the lung interstitium with an unclear cause and poor prognosis (1). It predominantly affects older adults, typically males aged 60–70 years, often with a history of smoking. The main clinical manifestations include exertional dyspnea and a persistent dry cough (2).

The chemotherapeutic agent bleomycin induces pulmonary fibrosis in 3–5% of treated patients, a side effect that has been widely used to develop animal models for studying the mechanisms of fibrosis (3-7). Genetic mutations, including those in the surfactant protein C gene, are associated with pulmonary fibrosis (8).

Oxidative stress is a hallmark of IPF. Patients exhibit impaired antioxidant capacity in both bronchoalveolar lavage fluid (BALF) and systemic circulation, along with marked depletion of intracellular glutathione (9,10). Elevated levels of oxidative stress biomarkers, including 8-isoprostane, are detected in BALF (11), and alveolar macrophages from patients with IPF generate increased superoxide anion (12). The inflammatory phase of pulmonary fibrosis involves inflammatory cell infiltration, vascular leakage, and pro-inflammatory cytokine release following epithelial injury (13).

Current evidence suggests that fibrosis is driven mainly by activated alveolar epithelial cells rather than chronic inflammation alone. These cells promote fibroblast and myofibroblast accumulation through mesenchymal cell proliferation, fibrocyte recruitment, and epithelial-to-mesenchymal transition. These fibroblast and myofibroblast aggregates produce excessive extracellular matrix, especially collagen, leading to irreversible lung scarring and structural distortion (14). Receptor tyrosine kinases have also been shown, in vitro and in animal models, to play critical roles in mediating these fibrotic processes (7). Despite advances in understanding the pathophysiology of IPF, IPF treatment remains palliative, highlighting the need for new therapies.

The multipathway nature of pulmonary fibrosis offers multiple potential therapeutic targets. Natural plant resources have attracted increasing attention as potential agents for pulmonary fibrosis, as they may act simultaneously on several pathological pathways, including oxidative stress, chronic inflammation, fibroblast activation, and collagen deposition, providing a comprehensive approach to disease management (15-19). In addition, these natural products often exhibit favorable safety profiles and can be sustainably sourced, supporting their use in preclinical and potentially clinical applications. Their traditional use in alleviating inflammation, pain, and respiratory disorders further supports their investigation in pulmonary fibrosis. Collectively, these characteristics make plant-based resources promising candidates for early therapeutic intervention and for the development of novel strategies to prevent or slow disease progression.

Acacia tortilis ssp. *raddiana* (*Acacia raddiana*) is a leguminous tree widely distributed in the Saharan and semi-arid regions of North Africa and the Sahel. *Acacia raddiana* has shown analgesic and anti-inflammatory properties (20-22), while the wood and roots are traditionally used to relieve dry cough (20,23,24). In some regions, preparations derived from *A. raddiana* are used to relieve symptoms associated with pulmonary conditions (25,26), suggesting potential anti-inflammatory and protective effects at the respiratory level.

Although *A. tortilis* ssp. *raddiana* is traditionally used for respiratory disorders in certain communities, no scientific study has validated its effects on pulmonary diseases. Therefore, this study aimed to evaluate the phytochemical composition, analgesic and anti-inflammatory activities, and early therapeutic potential in pulmonary fibrosis of *A. tortilis* ssp. *raddiana*.

Materials and Methods

Preparation of *Acacia raddiana* extract

Fresh leaves and gums of *A. raddiana* were harvested in southern Morocco and taxonomically identified by Professor Ahmed Ouhammou. A reference specimen (No. 13956) was archived in the herbarium of the Laboratory of Environment and Ecology. The leaves were dried, finely ground, and subjected to maceration in 70% ethanol at ambient temperature for 72 hours. After filtration, the solvent was removed under vacuum using a rotary evaporator. The aqueous residue was then freeze-dried at −85 °C under approximately 0.05 mbar for 24 hours, producing a dry extract yield of 14% (w/w). The gum was dried at ambient temperature, reduced to fine particles, and freshly suspended in distilled water each day before oral administration to the animals.

Detection of bioactive compounds

Alkaloids

Alkaloids were identified using two different reagents. Mayer's reagent was added to 6 mL of filtrate or aqueous gum solution. A yellow precipitate confirmed the presence of alkaloids. Dragendorff's reagent was added to 6 mL of filtrate or aqueous gum solution. A red precipitate indicated the presence of alkaloids (27).

Tannins

Tannins were detected by treating the filtrate or aqueous gum solution with ferric chloride reagent. Development of a dark blue or greenish-gray color indicated a positive result (28).

Coumarins

The presence of coumarins was detected by fluorescence under ultraviolet (UV) light after alkalinization of the extract. The extract was dissolved in distilled water, and 10% NH_4OH was subsequently added to the test solution.

The presence of coumarins was indicated by fluorescence emission under UV illumination, whereas no fluorescence was observed in the untreated control solution (29). Similarly, 1 mL of 10% ammonium hydroxide was added to the gum placed on filter paper, and fluorescence was subsequently observed under UV light. Fluorescence under UV light confirmed the occurrence of coumarins.

Flavonoids

The filtrate and gum were treated with sodium hydroxide, then hydrochloric acid was introduced into the mixture. The yellow color observed in the sodium hydroxide solution disappeared after dilute hydrochloric acid was introduced, confirming the occurrence of flavonoids (30).

Anthraquinone

The gum or dried leaf extract were initially treated with 10% ammoniacal solution (1 mL), followed by the addition of chloroform (2 mL). Formation of a pink to red coloration within the ammoniacal phase was indicative of anthraquinone derivatives (30).

Saponins

The filtrate (0.5 mL) was diluted with water and vigorously shaken. Similarly, the aqueous gum solution was stirred for 15 seconds. The persistence of foam for 10 minutes indicated the presence of saponins (28).

Steroidal and triterpenoid compounds

Steroidal and triterpenoid compounds were identified after treatment of the extract (5 mL) with chloroform (2 mL of chloroform) and concentrated sulfuric acid (3 mL). The formation of a reddish-brown coloration at the interface between the chloroform and sulfuric acid layers indicated the presence of steroids and triterpenes (31).

Quantitative analysis: Estimation of polysaccharide

The polysaccharide content of *A. raddiana* gum was estimated using a colorimetric method based on the phenol–sulfuric acid reaction (32). Initially, the blank mixture was obtained by introducing sulfuric acid into distilled water containing phenol reagent (5%). A glucose standard solution (100 µg/mL) was freshly prepared using distilled water as the solvent. Aliquots were then diluted to obtain final sugar concentrations ranging from 28 to 84 µg/mL. To each aliquot, phenol reagent (5%) and sulfuric acid were added, followed by a 10 minutes reaction period prior to absorbance reading at 490 nm using the blank as reference.

For the test sample, approximately 5 mg of *A. raddiana* gum was dissolved in 50 mL of distilled water. An aliquot of 1 mL of the solution was reacted with phenol reagent (5%) and sulfuric acid, then allowed to stand for 10 minutes prior to absorbance reading at 490 nm. Polysaccharide concentration was subsequently estimated using the calibration curve.

High-performance liquid chromatography (HPLC) profiling of the leaf extract

The extracts were analyzed by HPLC. Compound separation was achieved on an HPLC system fitted with a reversed-phase C18 column (4.6 µm, 2.1 × 250 mm). Elution was carried out using a solvent system composed of absolute methanol and aqueous formic acid (0.1%). The gradient elution program of absolute methanol in 0.1% aqueous formic acid (v/v) was as follows: 5% (0–3 min), 25% (3–6 min), 25% (6–9 min), 37% (9–13 min), 37% (13–18 min), and 54% (18–26 min).

Chromatographic analysis was performed at 25 °C using a mobile phase flow of 1 mL/min and a 20 µL injection volume to ensure optimal compound separation. The HPLC data were used to identify the compounds present in the prepared extract. Compound identification was performed by comparison with authentic standard compounds.

Thermal nociception assay

The assay followed the procedure reported by Oehme et al (33). Twenty-five male Swiss albino mice, food-deprived for 12 hours with water provided ad libitum, were allocated into four experimental groups and received oral treatment (p.o.): control group, saline (10 mL/kg, used as the vehicle); tramadol group, 0.04 g/kg; and *A. raddiana* extract-treated groups, 0.5 or 1 g/kg. The administered doses were chosen according to previous studies, which showed that *A. raddiana* leaf extract at 2 g/kg body weight caused no mortality or observable signs of toxicity in experimental animals (34). The tramadol dose (0.04 g/kg) was selected from previous rodent studies showing effective analgesia without major sedative effects (35).

Thermal nociception was evaluated using a heated plate adjusted to 55 ± 1 °C. The latency time (s) between first contact with the heated surface and the animal's first response (hind paw licking, jumping, or paw withdrawal) was recorded, with a cut-off time of 20 seconds. Baseline latency was measured 15 minutes before treatment, whereas response latency was recorded 30 minutes after treatment. Analgesic activity was calculated using the following equation:

$$\text{Maximum possible analgesia (\%)} = \frac{\text{Response latency- Basic latency}}{\text{Cut off time - Basic latency}} \times 100$$

Acetic acid–evoked abdominal constriction assay

Chemical-induced nociception was performed according to the previous method described (36). Following fasting, mice were assigned to four groups (n = 5):

- Control group: Mice received saline orally (0.1 mL/10 g body weight, p.o.) as the vehicle 30 minutes before acetic acid (2%, 0.1 mL/10 g body weight).
- Acetylsalicylic acid group: Mice received

acetylsalicylic acid (0.1 g/kg, p.o.) 30 minutes prior to acetic acid administration. Acetylsalicylic acid was used as a reference standard for peripheral analgesia in this model.

- *Acacia raddiana* extract-treated groups: Mice received the extract at doses of 0.5 or 1 g/kg (p.o.) 30 minutes preceding acetic acid treatment

In all groups, writhing episodes were counted for 30 minutes after acetic acid administration (2%).

Anti-inflammatory activity of the extract

The procedure of Sugishita et al (37) was used with minor modifications. Before the experiment, mice were deprived of food for 12 hours while water remained available ad libitum. The animals were randomly allocated into four groups (n = 5 per group) and received the following treatments:

- Control group: Mice received the vehicle orally (10 mL/kg, p.o.). After a 30 minutes treatment interval, a subplantar injection of 0.1 mL of 1% carrageenan was administered into the left hind paw. Paw volume was evaluated 1, 3, 5, and 24 hours following carrageenan administration.
- Indomethacin group: Mice received Indomethacin (0.01 g/kg, p.o.), followed by carrageenan injection and paw volume measurements as described for the control group.
- *Acacia raddiana* extract-treated groups: Mice received the extract orally at doses of 0.5 or 1 g/kg, followed by carrageenan injection and paw volume measurements as described for the control group.

The increase in paw volume was expressed as a percentage of the baseline value using the following formula:

$$\text{Change in paw volume (\%)} = \frac{(\text{Volume after carrageenan treatment} - \text{Basal volume}) \times 100}{\text{Basal volume}}$$

Early therapeutic effects of *Acacia raddiana* in pulmonary fibrosis

Induction of pulmonary fibrosis

Thirty male Swiss albino mice (8–10 weeks old, 28–36 g) were divided into six groups (n = 5) and housed under standard laboratory conditions with ad libitum access to food and water. Animals received intratracheal instillation of bleomycin (4 mg/kg) in saline or saline alone, as previously described (38).

- Control group: Mice received intratracheal saline followed, seven days later, by oral administration of saline (p.o.) once daily for 14 days.
- Bleomycin group: Mice received intratracheal bleomycin (0.004 g/kg) followed, seven days later, by oral administration of saline once daily for 14 days. The 0.004 g/kg intratracheal dose of bleomycin was selected from previous studies reporting reproducible induction of pulmonary inflammation and fibrosis with acceptable survival. Bleomycin was administered intratracheally under light anesthesia using a standardized procedure to ensure uniform pulmonary distribution and minimize experimental variability (39).
- Bleomycin + *Acacia raddiana* extract-treated groups: Seven days after bleomycin administration, mice received *Acacia raddiana* extract orally at doses of 0.5 or 1 g/kg (p.o.) once daily for 14 days.
- Bleomycin + *Acacia raddiana* gum-treated groups: Seven days after intratracheal bleomycin administration, mice received an aqueous gum solution of *Acacia raddiana* orally at doses of 0.5 or 1 g/kg (p.o.) once daily for 14 days. The doses of 0.5 and 1 g/kg were selected based on an acute toxicity study, which showed no mortality or toxic effects at 5,000 mg/kg, indicating that these doses were safe and suitable for biological evaluation (40).

At study termination, animals were first placed under anesthesia using chloral hydrate prior to euthanasia by cervical dislocation. The lungs were then excised for biochemical analysis and histological examination.

Biochemical analysis

Lung tissue malondialdehyde assay

Malondialdehyde (MDA) levels were determined using the method of Heath and Packer (41). Lung tissue was homogenized in trichloroacetic acid (10%, w/v) and acetone (90%, v/v). The use of trichloroacetic acid (TCA)–acetone in the sample preparation step serves to precipitate proteins and remove non-lipid interfering substances, thereby improving the specificity and reliability of the assay. The homogenate was centrifuged at 8,000 × g for 15 minutes, and 250 µL of the supernatant was collected and mixed with 0.5 mL of 0.1% (v/v) phosphoric acid and 0.5 mL of 0.6% (w/v) thiobarbituric acid. The reaction medium was heated at 100 °C for 30 minutes. After incubation, 1-butanol (0.75 mL) was added, and the preparation was recentrifuged at 8,000 × g. The absorbance of the butanol phase was then measured at 532 nm. Absorbance at 600 nm was also recorded to correct for interference from pigments or other substances absorbing at 532 nm. Lipid peroxidation levels were determined using the following equation:

$$\text{Malondialdehyde concentration} = \frac{(A532 - A600) \times 2 \times 2}{\varepsilon \times 1000 \times 0.25 \times m}$$

Where:

Malondialdehyde concentration was expressed as µM/g of lung tissue

$\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$

m = lung tissue weight (g)

Assay of peroxidase in lung tissue

Peroxidase activity was assessed following the method of Chance and Maehly (42). Briefly, 0.1 mL of lung tissue homogenate was added to a reaction mixture containing 2.9 mL of 50 mM phosphate buffer (NaH_2PO_4/Na_2HPO_4,

pH 7.4), 0.5 mL each of guaiacol reagent (0.05 M) and diluted H_2O_2 solution (0.03%). The assay was conducted at 25 ± 1 °C. Absorbance change was monitored at 470 nm over 3 minutes, and enzyme activity was calculated from the slope (ΔA/min) using the Beer–Lambert law. Peroxidase activity was reported as U/g of tissue.

Assay of catalase in lung tissue

Lung tissue samples were mechanically homogenized using a 50 mM phosphate buffer solution (pH 7.0) prepared from NaH_2PO_4 and Na_2HPO_4, then centrifuged to obtain the supernatant. The assay mixture consisted of 1.0 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of supernatant, and 0.4 mL of hydrogen peroxide solution (0.2 M). The reaction was conducted at 25 ± 1 °C. After incubation, 2.0 mL of dichromate–acetic acid reagent was added to terminate the reaction, followed by incubation at 100 °C for 10 minutes. Absorbance measurements were taken at a wavelength of 620 nm. Catalase activity was expressed as μmol of H_2O_2 decomposed per minute (μM/min) (42).

Histological analysis of mouse lungs

Lung samples were fixed in buffered formalin (10%) and paraffin-embedded. Sections (4 μm) were stained with H&E for histopathological examination and Masson's trichrome for collagen assessment.

Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple-comparison test in GraphPad Prism (version 9.0.0). Statistical significance was established at $P < 0.05$. Data are reported as mean values ± SEM.

Results

Bioactive compounds identified in *Acacia raddiana* leaf extract

Phytochemical profiling revealed flavonoids, saponins, tannins, anthraquinones, coumarins, steroids, and triterpenes in the leaf extract, while the gum contained mainly saponins, terpenes, sterols, and coumarins. HPLC analysis revealed rutin as the principal constituent of the leaf extract, accounting for 24% of the total identified compounds (Figure 1). The polysaccharide content of the gum was estimated to be 58.8% (w/w).

Thermal and chemical nociceptive responses in mice treated with *Acacia raddiana*

The results of the thermal stimulation test were expressed as the percentage of maximum possible analgesia. Administration of *A. raddiana* leaf extract (0.5–1 g/kg) significantly increased the percentage of maximum possible analgesia compared with the vehicle-treated group ($P < 0.01$), as shown in Table 1. The analgesic activity of the *A. raddiana* leaf extract was comparable to that of tramadol.

Acetic acid-induced nociceptive responses were characterized by an increased number of writhes, as shown in Table 1. The reference drug, Aspirin, significantly attenuated the nociceptive behavior induced by acetic acid ($P < 0.01$). Similarly, the *A. raddiana* extract (0.5–1 g/kg) significantly decreased writhing responses relative to untreated animals ($P < 0.001$ to $P < 0.0001$).

Acacia raddiana reduces carrageenan-evoked paw inflammation

As shown in Figure 2, intraplantar injection of carrageenan induced a significant increase in paw volume in the control group (175 ± 51% after 24 hours). Pretreatment

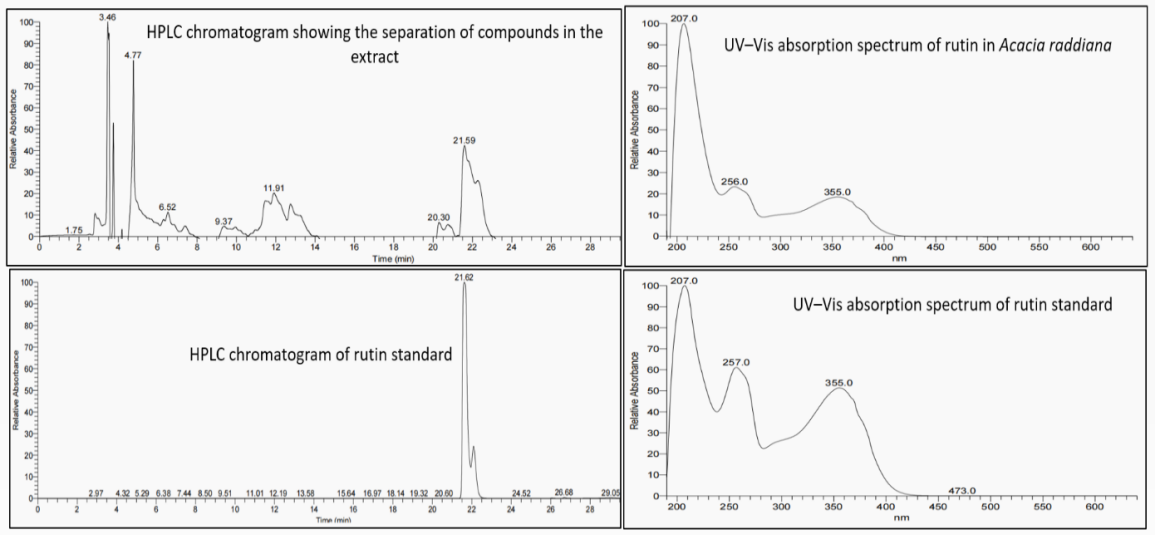

Figure 1. UV–Vis spectra and HPLC chromatograms of standard rutin and rutin identified in *Acacia raddiana* leaf extract.

Table 1. Effects of *Acacia raddiana* leaf extract on thermal and chemical nociceptive responses in mice

Tests	Parameters	Vehicle	Reference drug	*Acacia raddiana* leaf extract	
				0.5 g/kg	1 g/kg
Hot plate test	Percentage of maximum possible analgesia	17.28 ± 3.9	64.48 ± 9.8**	66.06 ± 9.4**	50.8 ± 3.4 **
Acetic acid-induced writhing test	Number of writhes	73.2 ± 7	28.8 ± 8**	18.2 ± 7***	14.2 ± 8****

The data are expressed as mean ± SEM. Statistical significance: **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ vs the vehicle-treated group. Vehicle: distilled water. Tramadol and acetylsalicylic acid served as reference drugs for thermal and chemical nociception tests, respectively.

with *A. raddiana* leaf extract and Indomethacin significantly inhibited paw edema throughout all phases of carrageenan-induced inflammation.

Early therapeutic effects of *Acacia raddiana* in experimental pulmonary fibrosis triggered by bleomycin

Changes in body weight in mice

A single intratracheal administration of bleomycin induced a significant loss of body weight. Treatment with *Acacia raddiana* markedly attenuated this effect (Figure 3).

Acacia raddiana attenuated bleomycin-induced oxidative stress in mouse lung tissue

All results are presented in Table 2. Bleomycin significantly increased malondialdehyde levels and peroxidase activity ($P < 0.0001$) and significantly decreased catalase activity compared with the control group ($P < 0.0001$).

Acacia raddiana leaf extract significantly reduced malondialdehyde levels and peroxidase activity compared with the bleomycin-treated group ($P < 0.0001$). At 0.5 g/kg, the extract further decreased catalase activity, whereas at 1 g/kg significantly increased catalase activity compared with bleomycin alone ($P < 0.0001$).

Acacia raddiana gum significantly decreased malondialdehyde levels ($P < 0.01$) and peroxidase activity ($P < 0.0001$) and significantly increased catalase activity ($P < 0.01$ and $P < 0.0001$ at 0.5 and 1 g/kg, respectively) compared with the bleomycin-treated group.

Acacia raddiana attenuated bleomycin-induced inflammatory cell infiltration in mouse lungs

No obvious infiltration of inflammatory cells was observed

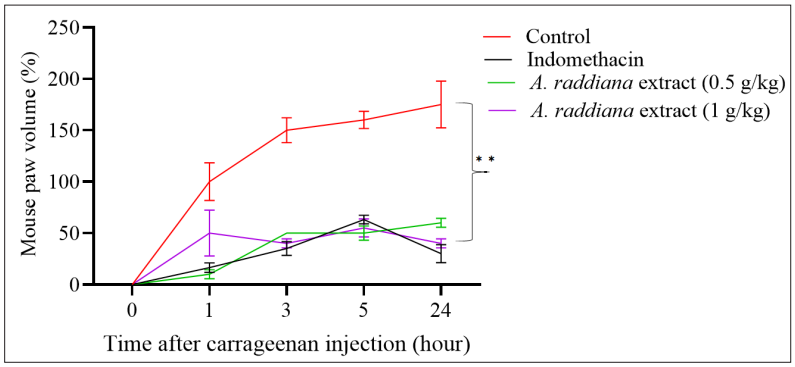

Figure 2. Anti-inflammatory effect of *Acacia raddiana* leaf extract in the carrageenan-induced paw inflammation. Significance level: **$P < 0.01$ relative to control. Paw edema was significantly inhibited by both the extract and indomethacin during the early and late phases of inflammation compared with the control group.

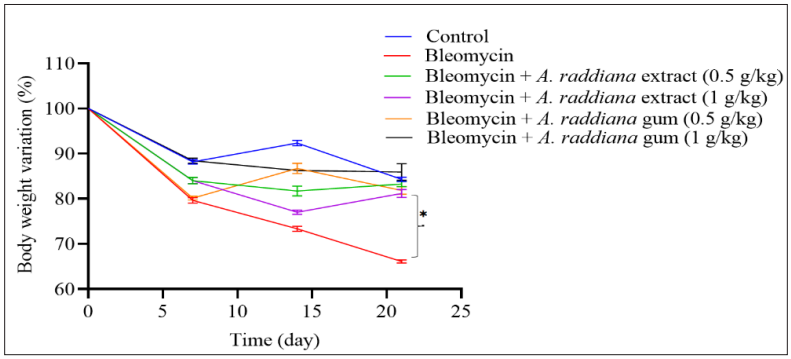

Figure 3. Body weight (%) of mice during the experimental period. Body weight variation (%) was determined using the following equation: (body weight on a given day × 100) / initial body weight. On day 21, all treated groups showed results that were significantly different from the bleomycin group, with $P < 5\%$.

Table 2. Reversal of bleomycin-induced oxidative stress in mouse lungs by *Acacia raddiana*

Experimental groups	Dose of gum/extract	Oxidative stress markers		
		Malondialdehyde levels (µmol/g tissue)	Catalase activity (µM of H_2O_2 degraded per minute)	Peroxidase activity (U/g tissue)
Control		24.71 ± 3.75	3689 ± 9.40	0.19 ± 0.002
Bleomycin		187.5 ± 3.09****	2748 ± 3.69****	0.46± 0.001****
Bleomycin + *Acacia raddiana* extract	0.5 g/kg	74.90 ± 0.67####	1893 ± 7.76####	0.24 ± 0.02####
	1 g/kg	63.98 ± 5.07####	3324 ± 2.12####	0.27 ± 0.008####
Bleomycin + *Acacia raddiana* gum	0.5 g/kg	28.54 ± 2.89##	3207 ± 9.09##	0.19 ± 0.00####
	1 g/kg	31.11 ± 6.49##	3579 ± 6####	0.20 ± 0.01###

Statistical significance is denoted as follows: ****$P < 0.0001$ vs control group; ##$P < 0.01$, ###$P < 0.001$ and ####$P < 0.0001$ vs bleomycin group. Malondialdehyde concentrations are expressed as µmol/g tissue. Peroxidase activity is expressed as U/g tissue. Catalase activity is expressed as µM of H_2O_2 degraded per minute (µM/min).

in the control group (Figure 4). Lung tissue sections from the bleomycin-treated group showed marked infiltration of inflammatory cells (green arrows) compared with the control group. These histopathological alterations were markedly attenuated by treatment with *A. raddiana* gum (0.5–1 g/kg) and *A. raddiana* leaf extract (1 g/kg). However, the 0.5 g/kg dose of the leaf extract failed to alleviate the severe infiltration of inflammatory cells.

Acacia raddiana attenuated bleomycin-induced collagen deposition in lung tissue

Lung tissue sections from the bleomycin-treated group showed excessive collagen deposition (red circle). This histopathological alteration was attenuated by treatment with *A. raddiana* (Figure 5).

Discussion

The anti-fibrotic potential of *A. raddiana* was investigated. Pulmonary fibrosis was induced in mice by a single intratracheal instillation of bleomycin. Three weeks after bleomycin administration, the results revealed advanced pulmonary fibrosis, as evidenced by increased malondialdehyde levels, altered catalase and peroxidase

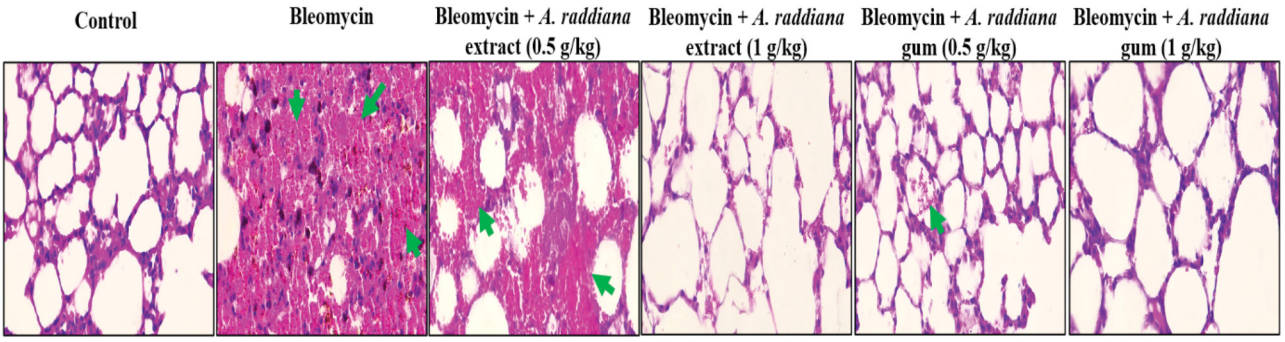

Figure 4. Hematoxylin and eosin-stained lung tissue sections from mice on day 21 post-intratracheal injection of saline or bleomycin (4 mg/kg), with or without oral treatment with *Acacia raddiana* (Magnification ×400). Bleomycin caused inflammatory cell infiltration (green arrows) compared with saline control, which was alleviated by *Acacia raddiana* gum (0.5–1 g/kg) and leaf extract (1 g/kg), but not by leaf extract at 0.5 g/kg.

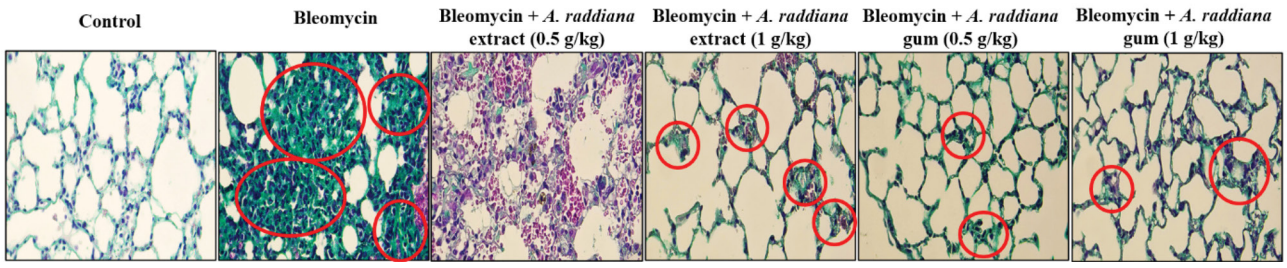

Figure 5. Collagen staining in lung sections using Masson's trichrome (×400). The red circle indicates collagen deposition. The Bleomycin group exhibited excessive collagen deposition.

activities, pronounced inflammation, and extensive collagen deposition. These findings are consistent with the well-established progression pattern of bleomycin-induced pulmonary fibrosis, which involves three stages: an early inflammatory phase (1–7 days after endotracheal bleomycin administration); a transitional phase characterized by gradual resolution of inflammation and maximal expression of interstitial collagen-related genes (7–14 days post-bleomycin); and a chronic phase marked by sustained collagen deposition during the third week after bleomycin administration (13,43,44). In addition, oxidative stress plays a key role in the pathophysiology of pulmonary fibrosis (10,12,44-48).

Treatment with *A. raddiana* was initiated on day 7 after bleomycin administration and continued once daily for 14 days. This treatment schedule allowed the evaluation of an early therapeutic intervention rather than a purely preventive effect. *A. raddiana* significantly attenuated fibrotic changes, suggesting that its anti-fibrotic activity may be mediated through antioxidant and anti-inflammatory mechanisms, as well as inhibition of collagen synthesis.

The 0.5 g/kg dose of *A. raddiana* leaf extract significantly reduced oxidative stress markers but did not markedly attenuate inflammatory cell infiltration in lung tissue. This apparent discrepancy may reflect a partial dissociation between oxidative stress and inflammatory cell recruitment in bleomycin-induced pulmonary fibrosis. Such dissociation has been previously reported, whereby oxidative injury may persist independently of inflammatory cell infiltration and may not be fully reversed by antioxidant interventions alone (4,49-51).

The anti-fibrotic activity observed in bleomycin-induced pulmonary fibrosis may result from the combined action of several classes of bioactive metabolites identified in both the leaf and gum extracts of *A. raddiana*. In the leaf extract, flavonoids are likely to be major contributors, particularly rutin, which was identified by HPLC as the principal constituent, accounting for 24% of the total identified compounds. Flavonoids, including rutin, have been reported to attenuate pulmonary fibrosis through antioxidant activity, Inhibition of fibroblast activation via suppression of transforming growth factor-β (TGF-β) signaling, and inhibition of collagen deposition (45). In the gum of *A. raddiana*, the high polysaccharide content (58.8% w/w) may play a central role in the observed protective effect, since plant polysaccharides are known to reduce oxidative stress, modulate immune responses, and suppress pro-fibrotic signaling pathways (52,53).

Pain and fibrosis are interconnected consequences of sustained tissue injury. While the analgesic effect of *A. raddiana* gum has been reported by Agouram et al (40), the present study evaluated the analgesic and anti-inflammatory properties of the leaf extract using three experimental models: thermal nociception test, abdominal constriction assay, and carrageenan-induced paw inflammation. The hot plate test is a standard method for assessing centrally acting analgesics (54). This model evaluates supraspinal nociception by measuring the latency of responses such as paw licking or jumping following thermal stimulation (55,56). Increased latency or percentage indicates central antinociceptive activity. Acute oral administration of *A. raddiana* leaf extract significantly increased analgesia in the hot-plate test, with effects comparable to tramadol, supporting a central analgesic effect potentially mediated via the opioidergic system. This central effect may be primarily attributed to flavonoids, particularly rutin, consistent with evidence that flavonoids can interact with opioid receptors and modulate nociceptive pathways. Indeed, flavonoids have been identified as potential opioid receptor ligands (57), and flavonoid-rich extracts have demonstrated naloxone-sensitive antinociceptive effects in experimental models (58).

The acetic acid writhing assay is widely used to assess the analgesic action of weak pain-relieving agents (59,60). Writhing results from prostaglandin-induced nociceptor sensitization. Pretreatment with *A. raddiana* leaf extract reduced acetic acid-induced writhing, suggesting inhibition of prostaglandin synthesis. This effect is most likely mediated by flavonoids, particularly rutin (24% of the extract), which are known to suppress prostaglandin synthesis through inhibition of cyclooxygenase activity and modulation of arachidonic acid metabolism (61).

Carrageenan-induced inflammation occurs in two phases. The initial inflammatory stage (0–1 h) primarily results from early liberation of histamine, serotonin, kinins such as bradykinin, and nitric oxide. This phase reflects an acute vascular response characterized by increased capillary permeability and edema formation, associated with mast cell activation and vasoactive mediators (62,63); The late phase (1–24 hours) involves the upregulation and activation of cyclooxygenase-2 and inducible nitric oxide synthase, leading to increased production of prostaglandins, pro-inflammatory prostanoids, and nitric oxide. This phase also includes the release of pro-inflammatory cytokines, neutrophil infiltration, and free radical generation, contributing to the amplification and maintenance of the inflammatory response (64,65). The leaf extract of *A. raddiana* significantly reduced carrageenan-induced edema, suggesting inhibition of prostaglandins, pro-inflammatory cytokines, nitric oxide production, and oxidative stress.

Limitations of the study

The use of crude leaf and gum extracts prevented precise attribution of the biological activities to individual constituents. In addition, molecular mechanisms were not fully explored, particularly key pathways such as TGF-β/Smad signaling, NF-κB signaling, and apoptotic markers,

which limit mechanistic interpretation of the anti-fibrotic effects.

Conclusion

Acacia tortilis ssp. *raddiana* exhibited notable analgesic and anti-inflammatory effects, reducing nociceptive responses and inhibiting carrageenan-induced edema. In the bleomycin-induced pulmonary fibrosis model, both the leaf extract and gum (0.5–1 g/kg) alleviated oxidative stress, inflammation, and collagen deposition. Phytochemical screening showed that the leaf extract contains flavonoids, saponins, tannins, anthraquinones, coumarins, steroids, and triterpenes, whereas the gum contains saponins, terpenes, sterols, and coumarins. Rutin was identified as the major constituent of the leaf extract (24%). The gum was rich in polysaccharides (58.8% w/w). These findings support the therapeutic potential of *A. raddiana* against pain, inflammation, and pulmonary fibrosis.

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Authors' contribution

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Conflicts of interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethical considerations

All experimental procedures were conducted in accordance with the European Community guidelines for the care and use of laboratory animals (Directive 86/609/EEC, 24 November 1986). Every effort was made to minimize animal suffering and reduce the number of animals used. The study protocol was approved by the Committee of Directors of the Faculty Research Laboratories (Approval No. BA-05/2025).

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References

1. Hennion N, Desseyn JL, Gottrand F, Wémeau-Stervinou L, Gouyer V. La fibrose pulmonaire idiopathique. Médecine/Sciences. 2022 ;38(6–7):6–7. doi:10.1051/medsci/2022084
2. Bonella F, Stowasser S, Wollin L. Idiopathic pulmonary fibrosis: Current treatment options and critical appraisal of nintedanib. Drug Des Devel Ther. 2015; 9:6407–19. doi:10.2147/DDDT.S76648
3. Walters DM, Kleeberger SR. Mouse models of bleomycin-induced pulmonary fibrosis. Curr Protoc Pharmacol. 2008;Chapter 5: Unit 5.46. doi:10.1002/0471141755.ph0546s40
4. Moeller A, Ask K, Warburton D, Gauldie J, Kolb M. The bleomycin animal model: A useful tool to investigate treatment options for idiopathic pulmonary fibrosis? Int J Biochem Cell Biol. 2008;40(3):362–82. doi:10.1016/j.biocel.2007.08.011
5. Liu T, De Los Santos FG, Phan SH. The bleomycin model of pulmonary fibrosis. Methods Mol Biol Clifton NJ. 2017; 1627:27–42. doi:10.1007/978-1-4939-7113-8_2
6. Adamson IY, Bowden DH. The pathogenesis of bleomycin-induced pulmonary fibrosis in mice. Am J Pathol. 1974;77(2):185–97.
7. Grimminger F, Günther A, Vancheri C. The role of tyrosine kinases in the pathogenesis of idiopathic pulmonary fibrosis. Eur Respir J. 2015;45(5):1426–33. doi:10.1183/09031936.00149614
8. Lawson WE, Polosukhin VV, Stathopoulos GT, Zoia O, Han W, Lane KB, et al. Increased and prolonged pulmonary fibrosis in surfactant protein C-deficient mice following intratracheal bleomycin. Am J Pathol. 2005;167(5):1267–77. doi:10.1016/S0002-9440(10)61214-X
9. Rahman I, Skwarska E, Henry M, Davis M, O'Connor CM, FitzGerald MX, et al. Systemic and pulmonary oxidative stress in idiopathic pulmonary fibrosis. Free Radic Biol Med. 1999;27(1–2):60–8. doi:10.1016/s0891-5849(99)00035-0
10. Mastruzzo C, Crimi N, Vancheri C. Role of oxidative stress in pulmonary fibrosis. Monaldi Arch Chest Dis. 2002;57(3–4):173–6.
11. Montuschi P, Ciabattoni G, Paredi P, Pantelidis P, du Bois RM, Kharitonov SA, et al. 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. Am J Respir Crit Care Med. 1998;158(5 Pt 1):1524–7. doi:10.1164/ajrccm.158.5.9803102
12. Schaberg T, Rau M, Stephan H, Lode H. Increased number of alveolar macrophages expressing surface molecules of the CD11/CD18 family in sarcoidosis and idiopathic pulmonary fibrosis is related to the production of superoxide anions by these cells. Am Rev Respir Dis. 1993;147(6 Pt 1):1507–13.

doi:10.1164/ajrccm/147.6_Pt_1.1507
13. Moore BB, Hogaboam CM. Murine models of pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2008;294(2): L152–60. doi:10.1152/ajplung.00313.2007
14. King TE, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet. 2011;378(9807):1949–61. doi:10.1016/S0140-6736(11)60052-4
15. Wang MC. Natural plant resource flavonoids as potential therapeutic drugs for pulmonary fibrosis. Heliyon. 2023;9(8):e19308. doi:10.1016/j.heliyon. 2023.e19308
16. Saadat S, Beigoli S, Khazdair MR, Amin F, Boskabady MH. Experimental and clinical studies on the effects of natural products on noxious agents-induced lung disorders, a review. Front Nutr. 2022; 9:867914. doi:10.3389/fnut.2022.867914
17. Wang L, Zhu T, Feng D, Li R, Zhang C. Polyphenols from Chinese herbal medicine: Molecular mechanisms and therapeutic targets in pulmonary fibrosis. Am J Chin Med. 2022;50(04):1063–94. doi:10.1142/S0192415X22500434
18. Hosseini S, Imenshahidi M, Hosseinzadeh H, Karimi G. Effects of plant extracts and bioactive compounds on attenuation of bleomycin-induced pulmonary fibrosis. Biomed Pharmacother Biomedecine Pharmacother. 2018; 107:1454–65. doi:10.1016/j.biopha.2018.08.111
19. Chen DQ, Feng YL, Cao G, Zhao YY. Natural products as a source for antifibrosis therapy. Trends Pharmacol Sci. 2018; 39(11):937–52. doi:10.1016/j.tips.2018.09.002
20. Bisht S, Kant R, Kumar V. α-d-Glucosidase inhibitory activity of polysaccharide isolated from *Acacia tortilis* gum exudate. Int J Biol Macromol. 2013;59:214–20. doi:10.1016/j.ijbiomac.2013.04.057
21. Lakhera AK, Kumar V. Monosaccharide composition of acidic gum exudates from Indian *Acacia tortilis* ssp. *raddiana* (Savi) Brenan. Int J Biol Macromol. 2017; 94:45–50. doi:10.1016/j.ijbiomac.2016.09.097
22. Agrawal NK, Gupta U, Kothari N, Chandra S, Singh R, Pandey S. Anti-nociceptive effect of seed extract of *Acacia tortilis* in rodents. Int J Basic Clin Pharmacol. 2018;7(4):650–4. doi:10.18203/2319-2003.ijbcp20181164
23. Hassan-Abdallah A, Merito A, Hassan S, Aboubaker D, Djama M, Asfaw Z, et al. Medicinal plants and their uses by the people in the Region of Randa, Djibouti. J Ethnopharmacol. 2013;148(2):701–13. doi:10.1016/j.jep.2013.05.033
24. Nguta JM, Mbaria JM. Brine shrimp toxicity and antimalarial activity of some plants traditionally used in treatment of malaria in Msambweni district of Kenya. J Ethnopharmacol. 2013;148(3):988–92. doi:10.1016/j.jep.2013.05.053
25. Abouri M, Mousadik AE, Msanda F, Boubaker H, Saadi B, Cherifi K. An ethnobotanical survey of medicinal plants used in the Tata Province, Morocco. International Journal of Medicinal Plant Research. 2012;1(7):99-123
26. Taha D, El Hajjaji S, Mourabit Y, Bouyahya A, Lee LH, El Menyiy N, et al. Traditional knowledge, phytochemistry, and biological properties of *Vachellia tortilis*. Plants. 2022; 11(23):3348. doi:10.3390/plants11233348
27. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry 2014;2(5):115-119.
28. Parekh J, Chanda S. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol. 2007 ;31(1):53-58.
29. N'Guessan K, Kadja B, Zirihi G, Traoré D, Aké-Assi L. Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). Sci Nat. 2009;6(1):1. doi:10.4314/scinat.v6i1.48575
30. Onwukaeme DN, Ikuegbvweha TB, Asonye CC. Evaluation of phytochemical constituents, antibacterial activities and effect of exudate of *Pycanthus angolensis* Weld Warb (Myristicaceae) on corneal ulcers in rabbits. Trop J Pharm Res. 2007;6(2):725–30. doi:10.4314/tjpr. v6i2.14652
31. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005;4(7):7. doi:10.5897/AJB2005.000-3127
32. Pawar HA, D'Mello PM. Spectrophotometric estimation of total polysaccharides in *Cassia tora* gum. J Appl Pharm Sci. 2011;1(3): 93-95.
33. Oehme P, Hilse H, Morgenstern E, Göres E. Substance P. Does it produce analgesia or hyperalgesia? Science. 1980; 208(4441):305–7. doi:10.1126/science.6154313
34. El-Ghazouani F, Amri O, Bouhaimi A, Zekhnini A. Myricitrin, kaempferol-3-O-rutinoside, and rutin from *Acacia tortilis* (Forssk.) Hayne ssp. *raddiana* alleviate liver injury in carbon tetrachloride (CCl4)-intoxicated rats. South Afr J Bot. 2025; 181:172–80. doi:10.1016/j.sajb.2025.04.024
35. Symeon I, Polissidis A, Balafas E, Stasinopoulou M, Alexakos P, Voyiatzaki C, et al. Evaluation of the effects of tramadol on analgesic response and locomotor activity on two different strains of laboratory mice. J Hell Vet Med Soc. 2017;68(1):89–96. doi:10.12681/jhvms.15567
36. Koster R, Anderson W, Beer E. Acetic acid for analgesic screening. Federation Proceedings. 1959 ;18 :412-418.
37. Sugishita E, Amagaya S, Ogihara Y. Anti-inflammatory testing methods: Comparative evaluation of mice and rats. J Pharmacobiodyn. 1981;4(8):565–75. doi:10.1248/bpb1978.4.565
38. Li B, Huang X, Liu Z, Xu X, Xiao H, Zhang X, et al. Ouabain ameliorates bleomycin induced pulmonary fibrosis by inhibiting proliferation and promoting apoptosis of lung fibroblasts. Am J Transl Res. 2018;10(9).
39. Kim SN, Lee J, Yang HS, Cho JW, Kwon S, Kim YB, et al. Dose-response effects of bleomycin on inflammation and pulmonary fibrosis in mice. Toxicol Res. 2010;26(3):217–22. doi:10.5487/TR.2010.26.3.217
40. Agouram F, Ezoubeiri A, Wafik A, Chait A, Sokar Z. Anti-nociceptive and antiurolithiatic activities of gum exudates from Moroccan *Acacia tortilis* ssp. *raddiana* (Savi) Brenan on ethylene glycol induced urolithiasis in rodents. J Ethnopharmacol. 2025;348:119819. doi:10.1016/j.jep.2025.119819
41. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 1968;125(1):189–98. doi:10.1016/0003-9861(68)90654-1
42. Maehly AC, Chance B. The assay of catalases and peroxidases. Methods Biochem Anal. 1954;1:357–424.

doi:10.1002/9780470110171.ch14
43. Chaudhary NI, Schnapp A, Park JE. Pharmacologic differentiation of inflammation and fibrosis in the rat bleomycin model. Am J Respir Crit Care Med. 2006; 173(7): 769–76. doi:10.1164/rccm.200505-717OC
44. Ma WH, Li M, Ma HF, Li W, Liu L, Yin Y, et al. Protective effects of GHK-Cu in bleomycin-induced pulmonary fibrosis via anti-oxidative stress and anti-inflammation pathways. Life Sci. 2020;241:117139. doi:10.1016/j.lfs.2019.117139
45. Bai L, Li A, Gong C, Ning X, Wang Z. Protective effect of rutin against bleomycin induced lung fibrosis: Involvement of TGF-β1/α-SMA/Col I and III pathway. BioFactors. 2020;46(4):637–44. doi:10.1002/biof.1629
46. Cameli P, Carleo A, Bergantini L, Landi C, Prasse A, Bargagli E. Oxidant/Antioxidant disequilibrium in idiopathic pulmonary fibrosis pathogenesis. Inflammation. 2020;43(1):1–7. doi:10.1007/s10753-019-01059-1
47. Otoupalova E, Smith S, Cheng G, Thannickal VJ. Oxidative stress in pulmonary fibrosis. Compr Physiol. 2020; 10(2):509–47. doi:10.1002/cphy.c190017
48. Oury TD, Thakker K, Menache M, Chang LY, Crapo JD, Day BJ. Attenuation of bleomycin-induced pulmonary fibrosis by a catalytic antioxidant metalloporphyrin. Am J Respir Cell Mol Biol. 2001;25(2):164–9. doi:10.1165/ajrcmb.25.2.4235
49. Cheresh P, Kim SJ, Tulasiram S, Kamp DW. Oxidative stress and pulmonary fibrosis. Biochim Biophys Acta. 2013;1832(7):1028–40. doi:10.1016/j.bbadis.2012.11.021
50. Della Latta V, Cecchettini A, Del Ry S, Morales MA. Bleomycin in the setting of lung fibrosis induction: From biological mechanisms to counteractions. Pharmacol Res. 2015; 97:122–30. doi:10.1016/j.phrs.2015.04.012
51. Kinnula VL, Myllärniemi M. Oxidant-antioxidant imbalance as a potential contributor to the progression of human pulmonary fibrosis. Antioxid Redox Signal. 2008; 10(4):727–38. doi:10.1089/ars.2007.1942
52. Schepetkin IA, Quinn MT. Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. Int Immunopharmacol. 2006;6(3):317–33. doi:10.1016/j.intimp.2005.10.005
53. Chen RR, Li YJ, Chen JJ, Lu CL. A review for natural polysaccharides with anti-pulmonary fibrosis properties, which may benefit to patients infected by 2019-nCoV. *Carbohydr Polym*. 2020;247:116740. doi:10.1016/j.carbpol.2020.116740
54. Núñez Guillén ME, da Silva Emim JA, Souccar C, Lapa AJ. Analgesic and anti-inflammatory activities of the aqueous extract of *Plantago major* L. Int J Pharmacogn. 1997; 35(2):99–104. doi:10.1076/phbi.35.2.99.13288
55. Chavan MJ, Kolhe DR, Wakte PS, Shinde DB. Analgesic and antiinflammatory activity of kaur-16-en-19-oic acid from *Annona reticulata* L. bark. Phytother Res PTR. 2012; 26(2):273–6. doi:10.1002/ptr.3544
56. Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE. Pain measurement: An overview. Pain. 1985; 22(1):1–31. doi:10.1016/0304-3959(85)90145-9
57. Katavic PL, Lamb K, Navarro H, Prisinzano TE. Flavonoids as opioid receptor ligands: Identification and preliminary structure-activity relationships. J Nat Prod. 2007; 70(8): 1278–82. doi:10.1021/np070194x
58. Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin S, Mohamad AS, et al. Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. Fitoterapia. 2008;79(7–8):557–61. doi:10.1016/j.fitote.2008.06.005
59. Ferreira SH, Vane JR. New aspects of the mode of action of nonsteroid anti-inflammatory drugs. Annu Rev Pharmacol Toxicol. 1974;14:57–73. doi:10.1146/annurev.pa.14.040174.000421
60. Berkenkopf JW, Weichman BM. Production of prostacyclin in mice following intraperitoneal injection of acetic acid, phenylbenzoquinone and zymosan: Its role in the writhing response. Prostaglandins. 1988;36(5):693–709. doi:10.1016/0090-6980(88)90014-7
61. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacol Rev. 2000;52(4):673–751.
62. Di Rosa M, Giroud JP, Willoughby DA. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol. 1971;104(1):15–29. doi:10.1002/path.1711040103
63. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin edema in rats. J Pharmacol Exp Ther. 1969;166(1):96–103.
64. Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, et al. Nitric oxide: A key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br J Pharmacol. 1996;118(4):829–38. doi:10.1111/j.1476-5381.1996.tb15475.x
65. Morris CJ. Carrageenan-induced paw edema in the rat and mouse. Methods Mol Biol. 2003;225:115–21. doi:10.1385/1-59259-374-7:115