Protective Effects of Walnut (*Juglans Regia* L.) Shell Extract Against Isoniazid-Induced Pulmonary Inflammation in Rats

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ABSTRACT

Isoniazid (INH) is a first-line antituberculosis drug that can induce oxidative stress and tissue damage. Walnut (*Juglans regia* L.) shell extract contains high levels of antioxidant compounds with potential therapeutic applications. To investigate the protective effects of walnut shell methanol extract against INH-induced pulmonary inflammation in rats. Thirty-two adult male Wistar-Albino rats (250-300 g) were randomly divided into four groups (n=8): Control (saline), INH (44 mg/kg), INH + walnut shell extract 100 mg/kg, and INH + walnut shell extract 250 mg/kg. All treatments were administered orally for 7 consecutive days. Lung tissues were analyzed for malondialdehyde (MDA), glutathione (GSH), and catalase (CAT) levels, accompanied by comprehensive histopathological examination. INH administration significantly increased MDA levels and decreased GSH content and CAT activity in lung tissue compared to controls ($p<0.05$). Walnut shell extract dose-dependently reduced MDA levels and restored GSH content and CAT activity. Histopathologically, INH induced interalveolar cellular infiltration and peribronchial lymphoid tissue hyperplasia. Walnut shell extract, particularly at 250 mg/kg, significantly preserved normal pulmonary architecture and attenuated inflammatory changes. Walnut shell methanol extract demonstrates significant protective effects against INH-induced pulmonary inflammation through antioxidant mechanisms, suggesting potential therapeutic value in preventing INH-associated pulmonary toxicity.

Keywords: Isoniazid, walnut shell, pulmonary inflammation, oxidative stress, antioxidant activity

1. INTRODUCTION

Tuberculosis (TB) remains a significant global health threat, representing one of the most serious bacterial infectious diseases affecting humanity throughout history. This disease, caused by *Mycobacterium tuberculosis*, primarily affects the lungs and can be fatal if left untreated. The advent of antituberculosis chemotherapy marked a pivotal milestone in modern medicine, transforming TB from a predominantly fatal disease to a treatable condition. Isoniazid (INH), one of the cornerstone drugs in tuberculosis treatment, is utilized both for treating active disease and as prophylactic therapy in high-risk individuals to prevent disease development [1].

INH exerts its antimycobacterial effect by targeting specific components of the *Mycobacterium tuberculosis* cell wall, thereby inhibiting bacterial growth and replication. This medication is typically administered in combination with other anti-tuberculosis agents to enhance therapeutic efficacy and reduce the risk of resistance development. Despite its proven efficacy in active tuberculosis treatment, the clinical utility of INH is significantly limited by its adverse effect profile [2,3]. The most commonly reported adverse effects associated with INH therapy include gastrointestinal disturbances, hepatotoxicity, peripheral neurotoxicity, and central nervous system-related complications [4]. These adverse effects can significantly complicate the treatment process and increase therapy-related morbidity, potentially leading to treatment discontinuation and therapeutic failure.

Oxidative stress emerges as one of the principal mechanisms underlying INH-induced cytotoxicity. This pathological condition occurs when excessive reactive oxygen species (ROS) are generated within cells, overwhelming the endogenous antioxidant defense systems and resulting in cellular damage to vital macromolecules[5]. Research has demonstrated that ROS production increases proportionally with the efficacy of antituberculosis drugs such as INH, perpetuating cellular damage and tissue injury [6].

In this context, natural plant extracts possessing antioxidant properties have gained considerable attention as potential therapeutic agents for preventing oxidative stress-mediated damage. These botanical compounds can neutralize harmful ROS effects, thereby potentially mitigating cellular damage during treatment regimens.

The walnut (*Juglans regia* L.) belongs to the Juglandaceae family and represents an economically important tree species cultivated worldwide, particularly in the Americas, Mexico, and Asia. In Turkey, walnut cultivation is predominantly concentrated in the Northeastern and Eastern Anatolia regions. Comprehensive physicochemical analyses have revealed that walnut components contain exceptionally high concentrations of fatty acids, proteins, phytosterols, and various phenolic compounds [7].

Walnut shell has been reported to possess high antioxidant content and demonstrate potent radical scavenging activity [8,9]. These remarkable properties have made walnut shell a subject of extensive research interest. The present study aims to investigate the protective efficacy of

walnut shell extract against INH-induced pulmonary injury, leveraging its rich bioactive compound profile.

2. MATERIALS AND METHODS

Experimental Protocol and Animals

The experimental design employed 32 adult male Wistar-Albino rats weighing 250-300 g, randomly allocated into four distinct groups (n=8 per group). Prior to experimental procedures, all animals underwent a 7-day acclimatization period to ensure adaptation to laboratory conditions. Extracts and pharmaceutical agents were prepared at appropriate concentrations and administered at literature-validated dosages for 7 consecutive days [3,10].

Group 1 (n=8) Isoniazid group: Animals received isoniazid (44 mg/kg) dissolved in physiological saline for 7 days via oral gavage.
Group 2 (n=8) Walnut shell methanol extract 100 mg/kg: Animals received walnut shell extract (100 mg/kg) dissolved in physiological saline for 7 days via oral gavage, administered concurrently with isoniazid.
Group 3 (n=8) Walnut shell methanol extract 250 mg/kg: Animals received walnut shell extract (250 mg/kg) dissolved in physiological saline for 7 days via oral gavage, administered concurrently with isoniazid.
Group 4 (n=8) Control group: Animals received physiological saline for 7 days via oral gavage.

The dosages of walnut shell extracts were selected based on literature-supported phenolic potency, reducing capacity, and antioxidant properties [11]. Following the 7-day treatment period, animals were sacrificed 24 hours post-final administration using high-dose anesthesia. Lung tissues obtained post-sacrifice were subjected to comprehensive biochemical and histological analyses.

Pharmaceutical Agents

Isoniazid (INH): The INH dosage selection (I.N.H. 300 mg Tablet, Koçak Pharma) was based on established literature protocols [10].

Preparation of Walnut (Juglans regia L.) Shell Extract

Walnuts were obtained from authorized vendors and cracked to extract the inner shells. The collected shells were pulverized using liquid nitrogen in a mortar to achieve fine powder consistency. Four hundred grams of pulverized walnut shell samples were subjected to methanol extraction using a shaking water bath for seven days (methanol 60°C, 200 ml ×8 extractions). The extracts were filtered, dried, and the well-drained methanol extract was administered to experimental animals.

Tissue Collection and Preparation for Biochemical Analyses

Following experimental procedures, animals were euthanized using high-dose anesthetic agents (xylazine hydrochloride, ketamine hydrochloride). Excised lung tissues were initially cleaned with physiological saline, with portions preserved in 10% buffered formaldehyde solution for histological examination. Remaining tissue samples were stored at -80°C for biochemical enzyme analyses.

Lung tissues were pulverized using liquid nitrogen in a mortar to achieve powder consistency. From each tissue sample, 0.5 g was weighed and 4.5 ml of appropriate buffer solutions (utilizing different buffer systems for each parameter) were added. Samples were homogenized using an ultra-turrax homogenizer for 10 minutes on ice. Homogenates were filtered through filter paper and centrifuged using a refrigerated centrifuge at literature-specified speeds at 4°C for each enzyme. The resulting clear supernatant was utilized for biochemical enzyme activity determinations.

Lipid peroxidation (LPO) and GSH level determinations in tissue homogenates were performed using the methods of Ohkawa et al. and Sedlak & Lindsay, respectively. Catalase (CAT) activity measurements were conducted according to Aebi's measurement principles [12-14].

3. RESULTS

Biochemical Parameters

INH administration resulted in a significant increase in MDA levels in lung tissue compared to the control group. A dose-dependent significant reduction in MDA levels was observed in the walnut extract-treated groups compared to both control and INH groups. GSH levels demonstrated significant reduction in the INH-treated group. While an increase was observed in the control group compared to INH treatment, more pronounced elevations were detected in walnut extract-treated groups. Examination of CAT activity in lung tissues revealed marked activity reduction in the INH-treated group, while both doses of walnut shell extract demonstrated significant activity enhancement (Table 1).

Histopathological Findings

Control group rats exhibited normal histological lung architecture with only mild alveolar hyperemia. INH-treated group rats demonstrated increased interalveolar tissue due to cellular infiltration and peribronchial lymphoid tissue hyperplasia. Walnut extract treatment, particularly at the 250 mg/kg dose, significantly preserved normal histological architecture. In the 100 mg/kg walnut methanol extract group administered with INH, interalveolar cellular infiltration-related thickening and peribronchial lymphoid tissue hyperplasia were relatively attenuated compared to the INH-only group. In the 250 mg/kg walnut methanol extract group administered with INH, lung alveoli predominantly maintained normal histological structure, with increased interalveolar cellular infiltration observed only in limited regions (Figure 1).

Table 1: Effects of walnut shell methanol extract (100 mg/kg and 250 mg/kg), isoniazid (400 mg/kg) and control groups on the amount of GSH and LPO, activities CAT in isoniazid -induced rat lung tissue.

Means in the same column by the same letter are not significantly different to the Duncan test P < 0.05). Mean

Treatment	LPO Level *(nmol/g tissue)*	CAT Activity *(nmol/min/mg tissue)*	GSH Level *(nmol/mg tissue)*
Isoniazid (400 mg/kg)	14.1±0.1d	131.0±0.4d	2.8±0.2a
Walnut shell methanol extract (100 mg/kg)	11.4±0.2c	122.7±1.0c	4.9±0.2b
Walnut shell methanol extract (250 mg/kg)	8.3±0.1b	111.0±0.5b	7.0±0.2c
Control	7.1±0.1a	99.8±0.6a	8.1±0.1d

damage index ± SE of eight animals in each group.

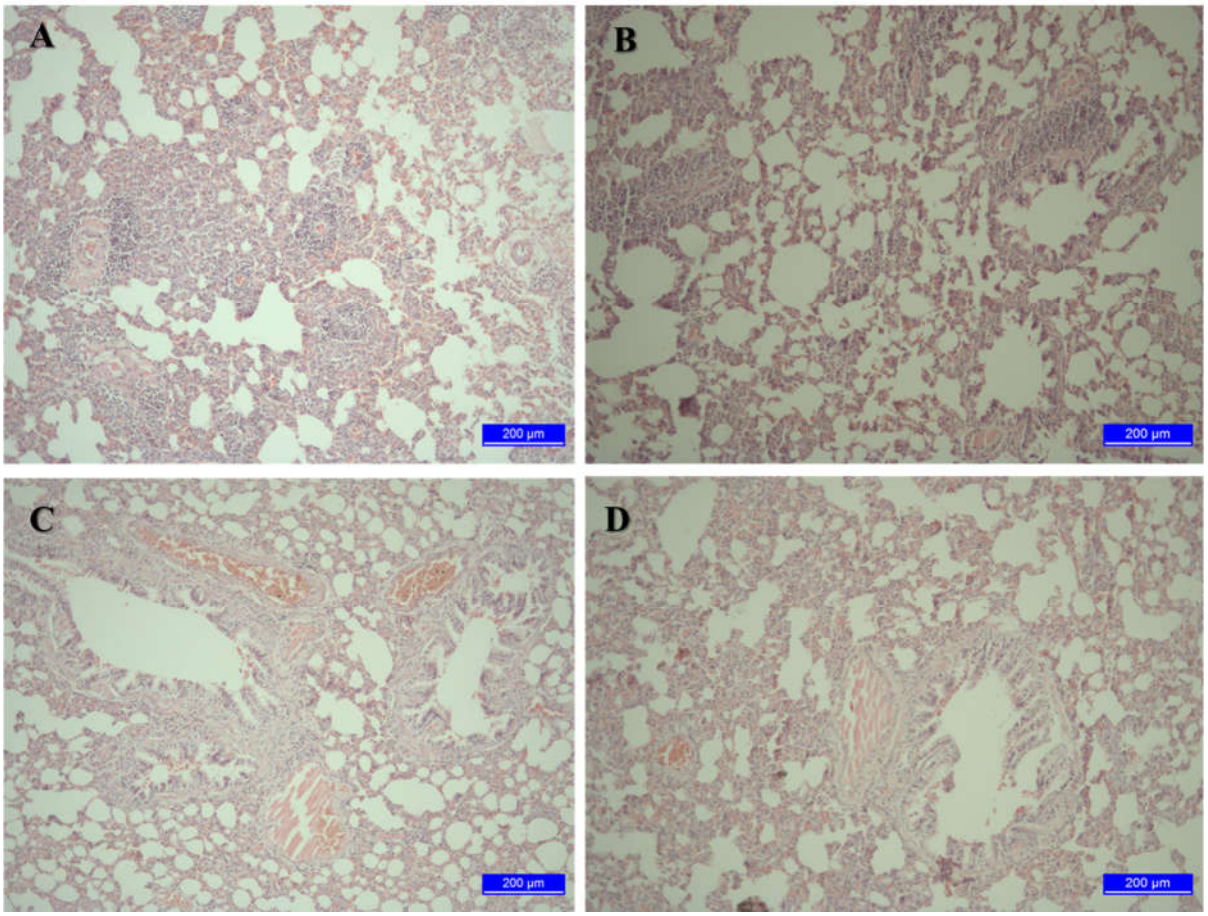

Figure 1: The isoniazid-treated rat lung tissues, (A-Control, B- isoniazid 400 mg/kg, C- Walnut shell methanol extract 100 mg/kg, D- Walnut shell methanol extract 250 mg/kg

4. DISCUSSION

This study investigated the protective effects of walnut shell methanol extract against INH-induced pulmonary injury. Our findings demonstrate that INH induces oxidative stress and inflammation in lung tissue, while walnut shell extract significantly attenuates these deleterious processes.

Despite being a first-line agent in tuberculosis treatment, INH carries substantial organ toxicity risks. INH toxicity is associated with reactive metabolites generated through metabolic activation, which form covalent bonds with cellular macromolecules, leading to mitochondrial dysfunction and oxidative stress [3]. During INH metabolism, reactive metabolites including hydrazine and acetylhydrazine are generated via CYP2E1 enzyme activity, contributing to increased oxidative stress [2].

The elevated MDA levels observed in our study align with literature findings demonstrating INH-induced oxidative stress enhancement. INH-related hepatic injury has been associated with oxidative stress, mitochondrial dysfunction, drug-metabolizing enzymes, and endoplasmic reticulum stress [15]. We determined that INH also exerts toxic effects in pulmonary tissue. Literature contains rare case reports describing INH-induced pulmonary fibrosis and interstitial lung disease [16].

The interalveolar cellular infiltration and peribronchial lymphoid tissue hyperplasia observed in our study indicate that INH induces inflammatory responses in pulmonary tissue. The increased MDA levels and decreased GSH content in the INH group demonstrate enhanced lipid peroxidation and compromised antioxidant defense systems, hallmarks of oxidative stress.

MDA represents a crucial biomarker resulting from ROS-mediated lipid attack and is utilized for oxidative stress assessment in various pathological conditions [17]. Catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) enzymes collectively constitute the primary defense system against ROS-induced oxidative stress [18]. The reduced CAT activity observed in our INH group indicates compromised antioxidant defense mechanisms.

Walnut shell extract has been reported to demonstrate protective effects against citric acid-induced pulmonary inflammation, with these effects associated with antioxidant enzyme system stimulation and reduced IL-6 concentrations [19]. *Juglans regia* kernel extract has shown protective effects against cigarette smoke-induced pulmonary toxicity, attributed to high polyphenol content [20]. *Juglans regia* extract has been demonstrated to reduce oxidative stress and pulmonary inflammation in bleomycin-induced lung injury while modulating alveolar macrophage inflammatory responses [21].

The more pronounced protective effect observed with the 250 mg/kg dose compared to 100 mg/kg in our study indicates dose-dependent antioxidant activity of walnut shell extract. Walnut shell extracts obtained using different solvents exhibit varying antioxidant activities, with ethyl acetate extract demonstrating the highest radical scavenging activity [8].

Various components of *Juglans regia* possess potent antioxidant activity with IC50 values against reactive oxygen species at μg/mL levels [22]. *Juglans regia* leaf extract has been shown to exert anti-inflammatory effects in diabetic nephropathy by inhibiting the NF-κB pathway [23].

The histopathological findings observed in our study, consistent with literature reports, demonstrate that walnut extract protects pulmonary tissue against inflammation. The interalveolar cellular infiltration and peribronchial lymphoid tissue hyperplasia observed in the INH group represent indicators of acute inflammatory response. The substantial preservation of histological architecture in walnut shell extract-treated groups, particularly at high doses, indicates the anti-inflammatory and tissue-protective effects of the extract.

Conclusion

This study demonstrates that walnut shell methanol extract exhibits significant protective effects against INH-induced pulmonary injury. This protective efficacy is characterized by improved oxidative stress parameters, enhanced antioxidant defense systems, and reduced histopathological damage. Our findings suggest that walnut shells, rather than being considered medical waste, can be valorized as valuable antioxidant resources.

However, comprehensive safety studies and detailed investigation of mechanistic pathways are required before clinical implementation. Future research should focus on identifying active compounds responsible for the protective effects, optimizing extraction methods, and conducting dose-escalation studies to establish therapeutic windows.

Ethics Committee Approval

This study was conducted in accordance with the decision of Giresun University Local Ethics Committee for Animal Experiments, dated 09.10.2023 and numbered 2023/2.

Conflict of Interest

The authors declare no conflict of interest regarding this manuscript.

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