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Protective Potential of Morin in Ifosfamide-Induced Lung Toxicity: Modulation of Oxidative Stress, Inflammation and Apoptosis Parameters

İfosfamid ile İndüklenen Akciğer Toksisitesinde Morin'in Koruyucu Potansiyeli: Oksidatif Stres, İnflamasyon ve Apoptoz Parametrelerinin Modülasyonu

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ABSTRACT

In this study, the protective effect of morin against lung toxicity induced by ifosfamide (IFO), a widely used drug in cancer treatment, was investigated. A total of thirty-five male Sprague-Dawley rats were randomly distributed into five experimental groups: Control, Morin (200 mg/kg), IFO and two different morin doses (IFO + Morin 100 mg/kg and IFO + Morin 200 mg/kg). Rats were given morin 100 mg/kg or 200 mg/kg for 2 days and on the second day, IFO 500 mg/kg was administered as a single dose. Markers of oxidative stress, inflammation, autophagy and apoptosis were analyzed using biochemical methods. According to the data obtained, IFO increased malondialdehyde (MDA) levels in lung tissue, while decreasing superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) activities and glutathione (GSH) levels. However, it was observed that morin administration decreased MDA levels and increased GSH levels and GPx, SOD, CAT activities. IFO administration inhibited the expression of B-cell lymphoma-2 (Bcl-2) and the nuclear factor erythroid 2-related factor 2 (Nrf-2) /heme oxygenase-1 (HO-1) pathway, while increasing levels of nuclear factor-kappa B (NF-κB), inducible nitric oxide synthase (iNOS), Bcl-2-associated x protein (Bax), and cysteine aspartate specific protease-3 (Caspase-3). However, morin decreased NF-κB and iNOS levels and inhibited inflammation by activating the Nrf-2/HO-1 pathway and prevented apoptosis by decreasing Bax and Caspase-3 and increasing Bcl-2. It also caused a decrease in Beclin-1 levels. According to the findings, IFO by affecting various signaling pathways in lung tissue, causing cellular damage, while morin demonstrated protective qualities against this damage.

Keywords: Apoptosis, ifosfamide, inflammation, lung toxicity, morin, oxidative stress

ÖZ

Bu çalışmada, kanser tedavisinde yaygın olarak kullanılan bir ilaç olan İfosfamid (IFO)'in neden olduğu akciğer toksisitesine karşı morin'in koruyucu etkisi araştırıldı. Toplam otuz beş erkek Sprague-Dawley sıçanı rastgele olarak beş deney grubuna ayrıldı: Kontrol, Morin (200 mg/kg), IFO ve iki farklı Morin dozu (IFO + Morin 100 mg/kg ve IFO + Morin 200 mg/kg) uygulandı. Sıçanlara 2 gün boyunca Morin 100 mg/kg veya 200 mg/kg verildi ve ikinci gün IFO 500 mg/kg tek doz olarak uygulandı. Oksidatif stres, inflamasyon, otofaji ve apoptoz belirteçleri biyokimyasal yöntemlerle analiz edildi. Elde edilen verilere göre, IFO akciğer dokusunda malondialdehit (MDA) seviyelerini artırırken, süperoksit dismutaz (SOD), katalaz (KAT), glutatyon peroksidaz (GPx) aktiviteleri ve glutatyon (GSH) seviyelerini azalttı. Ancak morin uygulamasının MDA düzeyini azalttığı, GSH seviyeleri ve GPx, SOD, KAT aktivitelerini arttırdığı gözlemlendi. IFO uygulaması, B-hücreli lenfoma-2 (Bcl-2) ve nükleer faktör eritroid 2 ile ilişkili faktör 2 (Nrf-2) /hem oksijenaz-1 (HO-1) yolunun ekspresyonunu inhibe ederken, nükleer faktör-kappa B (NF-κB), indüklenebilir nitrik oksit sentaz (iNOS), Bcl-2 ile ilişkili x proteini (Bax) ve sistein aspartat spesifik proteaz-3 (Kaspaz-3) seviyelerini artırdı. Ancak morin NF-κB ve iNOS seviyelerini azalttı ve Nrf-2/HO-1 yolağını aktive ederek inflamasyonu inhibe etti ve Bax ve Kaspaz-3'ü azaltıp Bcl-2'yi artırarak apoptozu engelledi. Ayrıca Beklin-1 düzeylerinde azalmaya neden oldu. Bulgulara göre, IFO akciğer dokusunda çeşitli sinyal yollarını etkileyerek hücresel hasara neden olurken, morin bu hasara karşı koruyucu özellik gösterdi.

Anahtar Kelimeler: Akciğer toksisitesi, apoptoz, ifosfamid, inflamasyon, morin, oksidatif stres

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INTRODUCTION

Cytostatics and anti-neoplastics are anticancer medications with potent modes of action.[1] The medication works by preventing DNA synthesis or interfering with cell division. Apoptosis results from cell damage that cannot be repaired. However, the primary goal is for the medication to predominantly target cancer cells.[2] Ifosfamide (IFO), an alkylating drug from the oxazaphosphorine class, is used in conjunction with other anticancer medications to treat a range of solid tumors, such as lymphoma, small cell lung cancer, testicular cancer, soft tissue sarcoma, osteosarcoma, bladder cancer, cervical cancer, and ovarian cancer.[3] Like other cytostatic cancer drugs, IFO has serious and life-threatening side effects that limit its therapeutic value.[4] Myelosuppression, interstitial pneumonia, hemorrhagic cystitis, alopecia, vomiting, nausea, and arrhythmia are among the common adverse effects of IFO.[5] The hazardous metabolites 4-hydroxycyclophosphamide, acrolein, and phosphoramide mustard are produced in the liver and, to a lesser extent, in the lung from IFO, an analog of cyclophosphamide.[6] Mechanisms of this toxicity may include disruptions in pro-oxidant/antioxidant balance, enhancement of inflammatory pathways and modulation of apoptotic signaling in pulmonary tissues.[7]

Due to the harmful consequences of chemotherapy medications used in cancer treatment, efforts to discover novel cancer drugs have surged recently.[1] Since ancient times, medicinal plants have been used as therapeutic agents in the treatment of various human diseases due to their antibacterial, antioxidant, anti-inflammatory, antiapoptotic and antiproliferative effects.[8] Flavanoids are polyphenolic compounds found in many fruits, vegetables and plant roots.[9] White mulberry, fig, and cranberry branches are rich in morin, a flavonoid molecule that works by lessening the harmful effects of anticancer medications on cancer cells.[10] Morin has several pharmacological properties such as reducing oxidative stress by scavenging free radicals, preventing apoptosis by reducing the release of apoptotic factors from mitochondria.[11] When these beneficial pharmacologic effects of morin are evaluated, it can be considered that morin can be used for protective administrationtion in lung injury after IFO use. Nevertheless, there is no research on morin's protective properties against in the lung IFO damage.

In this study, biochemical parameters such as oxidative stress, inflammation, DNA damage, and apoptosis were analyzed to evaluate the potential protective effects of morin against IFO-induced pulmonary injury in male rats.

MATERIALS AND METHODS

Chemicals

IFO (Holoxan) was obtained through Eczacıbaşı, Istanbul, Turkey. Morin and other chemical reagents were obtained from Sigma-Aldrich, St. Louis, Missouri, USA.

Animals

Thirty-five male Sprague Dawley rats, weighing 200-250 g, were utilized in this investigation. They were acquired from the Erzurum Atatürk University Experimental Animals Center. Rats were housed in a room temperature of 24-25°C and a humidity of 45 ± 5%. The rats were given to adapt to the environment for one week before starting the experiment and were given as much water and standard food as they wanted throughout the experiment.

Experimental Design

Rats were split into five groups at random, each with seven rats:

Group I (Control): For two days, the rats were given oral saline; as a control, they were given a single dose of normal saline (1 mL i.p) on day two.

Group II (Morin): Rats were orally administered morin (200 mg/kg/day) for two days.[5]

Group III (IFO): Rats were given oral saline for two days, followed by a single dosage of IFO (500 mg/kg, i.p.) on day two to cause lung damage.[12]

Group IV (IFO+Morin 100): Rats were administered morin (100 mg/kg/day; p.o.) orally for two days and given a single dose of IFO (500 mg/kg) intraperitoneally on day two.

Group V (IFO+Morin 200): Rats were administered morin (200 mg/kg/day; p.o.) orally for two days and given a single dose of IFO (500 mg/kg, i.p.) intraperitoneally on day two.

24 hours following the final dose, the rats were killed under sevoflurane anesthesia was administered. The obtained lung tissues were preserved at -80°C for biochemical analyses.

Lipid Peroxidation and Antioxidant Activity Measurements

Lung tissue homogenates, needed to evaluate oxidative stress markers, were prepared according to the methodology indicated in our previous study.[13] Supernatants of lung tissue homogenates were analyzed for glutathione (GSH) levels by the Sedlak & Lindsay method,[14] for catalase (CAT) activity by the Aebi method,[15] for superoxide dismutase (SOD) activity by the Sun et al. method,[16] for glutathione peroxidase (GP_X) activity by the Lawrence and Burk method,[17] for malondialdehyde (MDA) content by the Placer et al. method,[18] and for total protein analysis by the Lowry et al. method.[19]

Determination of ELISA Markers

Nuclear factor-kappa B (NF-κB) (Cat. No: 201-11-0288), nuclear factor erythroid 2-related factor 2 (Nrf2) (Cat. No: 201-11-5375), heme oxygenase-1 (HO-1) (Cat. No: 201-11-0677), cysteine aspartate specific protease-3 (Caspase-3) (Cat. No: 201-11-5114), B-cell lymphoma-2 (Bcl-2) (Cat. No: 201-11-0038), Bcl-2-associated x protein (Bax) (Cat. No: 201-11-0035), inducible nitric oxide synthase (iNOS) (Cat. No: 201-11-0741), and Beclin-1 (Cat. No: 201-11-1689) (Sunred Biological Technology, Shanghai, China) were measured in lung tissue using rat ELISA kits. A measurement of absorbance was made at 450 nm.

Statistical Analysis

The Tukey test was used to identify group differences, while the one-way analysis of variance (ANOVA) test was employed to establish statistical differences and significant levels. All values are given as mean ± standard error (±SEM), while results at (P < .05) were considered significant. SPSS 20.0 (IBM SPSS Corp., Armonk, NY, USA) package program was used for these statistical analyses.

RESULTS

The effects of morin against the oxidative damage caused by IFO in the lung tissue were evaluated by oxidant marker MDA, antioxidant enzymes SOD, CAT, GPx activities and non-enzymatic marker GSH analysis and the findings are presented in Table 1. When we examined oxidative stress and lipid peroxidation in IFO-induced lung injury, we found that IFO significantly increased MDA levels and decreased SOD, CAT, GPx and GSH levels compared to control and morin groups (P < .05). On the other hand, IFO+Morin100 and IFO+Morin200 administration combined with IFO was found to decrease MDA levels while significantly increasing SOD, CAT and GPx activities and GSH levels (P < .05). It was determined that IFO+Morin200 was more effective in increasing CAT, GSH, and GPx levels compared to IFO+Morin100 (P < .05). However, there was no significant difference in SOD levels between the IFO+Morin100 group and the IFO+Morin200 group (P > .05).

Table 1. Effect of IFO and Morin on lung tissue MDA and GSH levels, SOD, CAT and GPx enzyme activities in each group.

Parameters	Control	Morin	IFO	IFO+Morin100	IFO+Morin200
MDA (nmol/g tissue)	22.20±0.48^{a}	23.14±0.55^{ab}	35.19±0.66^{d}	29.15±0.54^{c}	24.93±0.38^{ab}
GSH (nmol/g tissue)	1.81±0.04^{d}	1.85±0.02^{d}	0.77±0.02^{a}	1.04±0.03^{b}	1.45±0.02^{c}
SOD (U/g protein)	18.83±0.30^{c}	19.29±0.45^{c}	9.11±0.26^{a}	13.80±0.28^{b}	15.17±0.44^{b}
CAT (catal/g protein)	32.50±0.63^{d}	32.78±0.69^{d}	19.64±0.38^{a}	23.43±0.37^{b}	28.70±0.35^{c}
GPx (U/g protein)	26.83±0.62^{d}	27.59±0.58^{d}	15.82±0.32^{a}	19.64±0.25^{b}	24.11±0.44^{c}

Each group's values are given as the mean ± S.E.M. of seven rats. Significant differences between each group are indicated by different superscripts (a–d) in the same row (P < .05). MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; IFO: ifosfamide.

Evaluation of Inflammation Markers

To investigate the effect of morin administration on IFO-triggered inflammatory response, NF-κB, Nrf-2, HO-1 and iNOS levels in lung tissue were evaluated and presented in Figure 1. Compared to the control and morin groups, NF-κB (Figure 1a), Nrf-2 (Figure 1b) and iNOS (Figure 1d) levels increased, while HO-1 (Figure 1c) levels decreased in the IFO group (P < .05). These levels were found to decrease at doses of 100 and 200 mg/kg morin administered together with IFO (P < .05).

Evaluation of Apoptosis Markers

To investigate the biomolecular mechanisms of the anti-apoptotic effects of morin on IFO-induced apoptosis in lung tissue, protein levels of proapoptotic Bax and antiapoptotic Bcl-2 levels, and Caspase-3 were investigated and presented in Figure 2. According to the data, IFO down-regulated Bcl-2 (Figure 2b), while increasing Bax (Figure 2a) and Caspase-3 (Figure 2c) levels (P < .05). Following morin administration, it was found that Bcl-2 was elevated to prevent IFO-induced apoptosis and that Bax and Caspase-3 were inhibited. Furthermore, the findings suggest that high doses of morin provide a more pronounced protective effect on apoptosis (P < .05).

Evaluation of Autophagy Marker

When beclin-1 levels, the most important autophagy marker, were examined, it was found that Beclin-1 levels increased in the lung tissue in the IFO-administration group (P < .05) and 100 and 200 doses of morin administered together with IFO decreased Beclin-1 levels and suppressed autophagy (P < .05). Beclin-1 levels results are given in Figure 3.

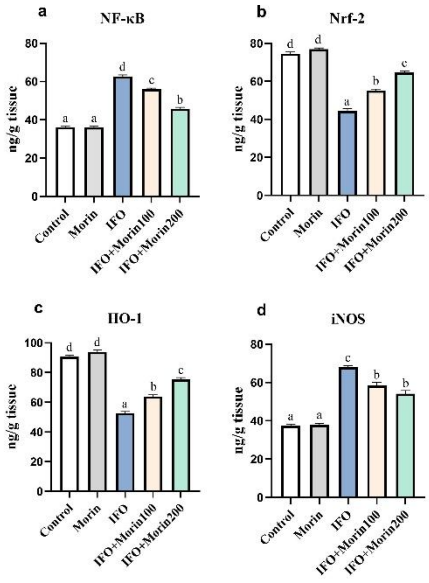

Figure 1. Lung tissue NF-κB (a), Nrf-2 (b), HO-1 (c), iNOS (d) levels after IFO and Morin administrations to rats. Each group's values are given as the mean ± S.E.M. of seven rats. Significant differences between each group are indicated by different superscripts (a–d) in the same row ($P < .05$).

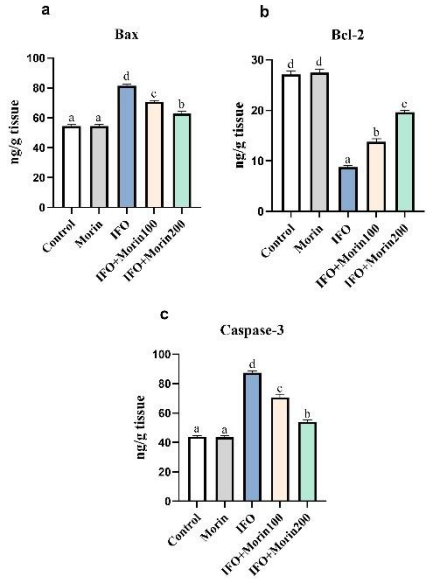

Figure 2. Lung tissue Bax (a), Bcl-2 (b) and Caspase-3 (c) levels after IFO and Morin administrations to rats. Each group's values are given as the mean ± S.E.M. of seven rats. Significant differences between each group are indicated by different superscripts (a–d) in the same row ($P < .05$).

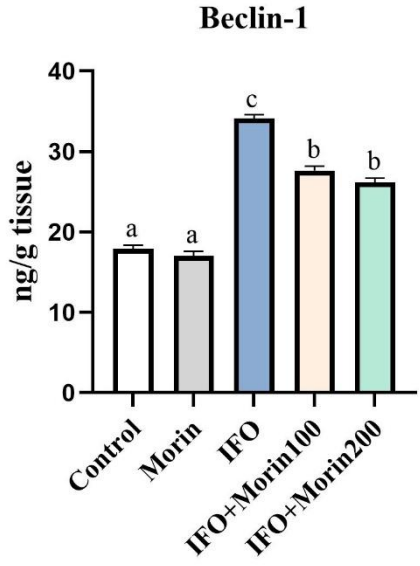

Figure 3. Lung tissue Beclin-1 levels after IFO and Morin administrations to rats. Each group's values are given as the mean ± S.E.M. of seven rats. Significant differences between each group are indicated by different superscripts (a–d) in the same row ($P < .05$).

DISCUSSION

With the use of antineoplastic agents in cancer treatment, the possible toxicity of these agents has also started to be evaluated.[20] Most antineoplastic agents have the potential to cause pulmonary toxicity by primarily affecting the lung parenchyma. In contrast, the airways, pleura and pulmonary circulatory system are more rarely affected.[21]

IFO, a cyclophosphamide analog, is commonly used to treat non-small cell lung cancer.[22] Although there have been several studies on the toxicity of IFO,[23, 24] lung injury has not been adequately described. Morin, an important flavonoid, has been shown to contribute to biological processes with its anti-inflammatory, antioxidant and anticancer properties. These effects were demonstrated by focusing on the detrimental effects of IFO on lung tissue via oxidative stress and molecular pathways and by examining the potential protective role of morin on these pathways.

The mechanism of toxicity for many substances, including chemotherapeutic medicines, is based on oxidative stress.[24] Reactive oxygen species (ROS) or reactive nitrogen species rise as a result, causing tissue damage.[25,26] The increase of free radicals triggers lipid peroxidation by damaging lipids, which are essential components of the cell membrane.[27] MDA is the most prominent indicator of lipid peroxidation caused by oxidative stress.[28] Previous studies showed that MDA levels rose when IFO was administered and fell after antioxidant therapy was received.[12,29] In line with recent studies, our findings demonstrated that IFO administration led to elevated MDA levels, indicating enhanced oxidative stress and associated cellular damage.

IFO is metabolized by the cytochrome P450 3A4 enzyme, and the resulting metabolites are highly toxic. Cytotoxic nitrogen mustards with potent electrophilic qualities, acrolein, and chloroacetaldehyde are among these metabolites. GSH, an important antioxidant in cells, is rapidly depleted by isophosphoramide mustard, acrolein and chloroacetaldehyde, leading to toxic effects.[30] According to Çakmak et al., IFO caused oxidative stress in testicular tissue by inhibiting a number of antioxidant enzymes, such as SOD, CAT, and GPx, as well as non-enzymatic antioxidants, such GSH.[24] In another investigation, it was found that administering IFO raised MDA levels while lowering GSH levels.[31] In our study, it was determined that IFO application suppressed the antioxidant defense system by causing a decrease in SOD, CAT and GPx enzyme activities and GSH levels, and thus increased oxidative stress damage. Morin administered as a protective has been reported to alleviate oxidative stress with its antioxidant properties.[9,32] In the present study, morin was found to reduce oxidative stress-induced damage by increasing the activities of SOD, CAT, and GPx, as well as GSH levels, while decreasing MDA levels. Notably, morin administered at a dose of 200 mg/kg was observed to ameliorate oxidative damage more effectively.

Nrf-2 protects against oxidative damage and inflammation.[33] In healthy cells, Nrf-2 and kelch-like ECH-associated protein 1 (Keap1) form heterodimer. Nrf-2 and Keap1 separate when any damage is caused by different stimuli, and Nrf-2 is then carried to the nucleus. Once in the nucleus, HO-1 activates transport molecules, cellular antioxidants such as NADPH quinine oxidoreductase-1, glutamate-cysteine ligase modifier subunit and glutamate-cysteine ligase catalytic subunit.[34] A previous study by Han et al. reported that IFO administration down-regulated Nrf-2-mediated oxidative stress response pathways.[33] According to another study, chemotherapeutic agents decreased Nrf-2 expression.[35] In our study, it was observed that IFO suppressed Nrf-2 levels, reduced HO-1 expression in lung tissue, and consequently induced cellular damage due to oxidative stress. However, there is evidence that plant-derived compounds activate the Nrf-2/HO-1 pathway.[9,11] Our research revealed that the Nrf-2/HO-1 pathway, which is suppressed by IFO in lung tissue, is activated by morin, a plant-derived compound, and exhibits a protective effect against oxidative stress.

A crucial transcription factor, NF-κB controls inflammatory and immunological responses and enhances cell viability by shielding cells from apoptosis.[36] NF-κB is normally kept inactive in the cytoplasm by the inhibitory proteins IκB. However, after phosphorylation and proteasomal degradation of IκB, NF-κB is activated and translocated to the nucleus. There, it guides the immune response by promoting the production of cytokines that are essential for controlling the inflammatory response, like tumor necrosis factor (TNF-α) and interleukin-1β.[37] Research indicates that TNF-α is a cytokine that is essential for controlling the expression of iNOS in inflammatory situations.[38] Studies have reported that IFO causes inflammation by activating NF-κB and iNOS expression.[39,40] Bachewal et al. reported that morin administration markedly decreased NF-κB expression. Furthermore, it was reported that this decrease suppressed the inflammatory cascade together with the decrease in iNOS expression and thus morin provided a strong anti-inflammatory effect.[41] In the presented study, it was observed that IFO administration triggered the inflammatory response by increasing the expression of NF-κB and iNOS, whereas morin administration reduced inflammation by decreasing the expression levels of these molecules.

Apoptosis is a mechanism of cellular death triggered by increased levels of oxidative stress. Increased ROS levels trigger proapoptotic genes including NF-κB and TNF-α, which start the cell death process.[11,38] In this process, members of the Caspase family play a key role because they regulate cell death by being directly or indirectly involved in all stages of apoptosis.[42] The mitochondrial route is another pathway that contributes to apoptosis. Disruption of the Bax/Bcl-2 balance triggers the release of cytochrome c (CytC) from the mitochondrial space into the cytoplasm, initiating activation of the apoptosis pathway.[43] CytC, which enters the cytoplasm in the presence of adenosine triphosphate, binds to apoptotic protease activating factor-1 (Apaf-1) and activates it.[44] Activated Apaf-1 attaches itself to the cysteine aspartate specific protease-9 (Caspase-9) precursor form and causes it to become active. The apoptosis process is started when active Caspase-9 activates Caspase-3.[45] Bcl-2 exhibits an anti-apoptotic impact by preventing the activation of proapoptotic proteins, whereas Bax promotes apoptosis by eliminating growth factors.[9] IFO administration was found to initiate the apoptotic process by raising Bax and Caspase-3 levels while lowering Bcl-2 levels in a prior work on brain tissue.[5] In testicular damage, morin, a flavonoid, has been shown to decrease the expression of Bax and Caspase-3, increase the expression of Bcl-2, and prevent apaptosis.[46] In our current study, it was observed that Bcl-2 levels decreased and Caspase-3 and Bax levels increased in the lung tissue of rats exposed to IFO. These findings suggest that apoptosis may play an important role in IFO-induced lung injury. However, it was determined that morin administration had a protective effect in preventing mitochondrial apoptosis in rats exposed to IFO.

A cellular mechanism called autophagy uses lysosomes to eliminate aging and damaged organelles.[47] Beclin-1 and the anti-apoptotic protein Bcl-2 combine to inhibit autophagy.[48] However, if beclin-1 is overproduced or Bcl-2 levels are reduced, Bcl-2 cannot bind to beclin-1 and autophagy is activated.[13] According to a previous study, a significant increase in beclin-1 protein levels was detected in the testicular tissue of rats given IFO.[24] According to an in vitro study on chronic obstructive pulmonary disease, morin inhibited autophagy by inhibiting beclin-1 levels.[49] In our current study, in accordance with the literature, it was observed that IFO induced autophagy by increasing Beclin-1 levels, whereas morin administration provided a protective effect against autophagy by decreasing Beclin-1 levels.

As a result, this study shed light on the potential pathways behind the protective effects of morin on lung tissue in IFO-induced toxicity. Due to excessive ROS production and links to inflammation, oxidative damage, apoptosis and autophagy, IFO use causes lung toxicity. According to the findings of this study, simultaneous supplementation of morin provides a protective effect on the lung tissues of rats by reducing oxidative damage, inflammation, apoptosis and autophagy.

Ethics Committee Approval: The animal experimentation procedure was approved by the Animal Experiments Local Ethics Committee of Ataturk University Date: 31.01.2025, Approval No: 2025-31/26).

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