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1 **Title:**

2 **Cadmium-induced lung injury is associated with oxidative stress, apoptosis and altered**
3 **SIRT1 and Nrf2/HO-1 signaling; protective role of the melatonin agonist agomelatine**

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23

24 **Abstract**

25 Cadmium (Cd) is a hazardous heavy metal extensively employed in manufacturing polyvinyl
26 chloride, batteries, and other industries. Acute lung injury has been directly connected to Cd
27 exposure. Agomelatine (AGM), a melatonin analog, is a drug licensed for treating severe
28 depression. This study evaluated the effect of AGM against Cd-induced lung injury in rats. AGM
29 was administered in a dose of 25 mg/kg/day orally, while CdCl₂ was injected intraperitoneal in a
30 dose of 1.2 mg/kg to induce lung injury. Pre-treatment with AGM remarkably ameliorated Cd-
31 induced lung histological abrasions. AGM decreased reactive oxygen species (ROS) production,
32 lipid peroxidation, suppressed NDAPH oxidase, and boosted the antioxidants. AGM increased
33 Nrf2, GCLC, HO-1, and TNXRD1 mRNA, as well as HO-1 activity and downregulated Keap1.
34 AGM downregulated Bax and caspase-3 and upregulated Bcl-2, SIRT1 and FOXO3 expression
35 levels in the lung. In conclusion, AGM has a protective effect against Cd-induced lung injury via
36 its antioxidant and anti-apoptotic effects mediated via regulating Nrf2/HO-1 and SIRT1/FOXO3
37 signaling.

38 **Keywords:** Agomelatine; Cadmium; Oxidative stress.

39 **Introduction**

40 Cadmium (Cd), a hazardous heavy metal, has been extensively employed in manufacturing
41 polyvinyl chloride, batteries, and others (Ashizawa et al., 2012, Fulcher and Gibb, 2014). Smoking
42 causes cadmium oxide to be deposited inside the lung or absorbed into the systemic circulation.
43 As a result, a smoker's body has a Cd level about three times greater than non-smokers (Pappas,
44 2011). Although cigarette smoke is the main cause of Cd toxicity in humans, Cd pollution of water,
45 air, and food also negatively influences human health (Rani et al., 2014). Acute lung injury has

46 been directly connected to Cd exposure (Li et al., 2018). Studies showed that Cd promotes
47 oxidative stress (OS) and inflammation, which harms the lungs and induces the release of pro-
48 inflammatory mediators by lung epithelial cells and the breakdown of extracellular matrix (Larson-
49 Casey et al., 2020). OS mediated via excess reactive oxygen species (ROS) is linked to Cd-induced
50 tissue damage and cell death. Chronic exposure to Cd leads to an imbalance of oxidants and
51 antioxidants since it increases ROS production and lowers antioxidant levels (Sarkar et al., 2013,
52 Rani et al., 2014). OS in the lung includes NADPH oxidases (NOXs)-mediated ROS generation
53 (Lee and Yang, 2012). Excess ROS provokes inflammation and both orchestrate cell death via
54 apoptosis (Shore and Nguyen, 2008). Therefore, attenuation of these pathologic processes can
55 effectively protect the lung against Cd-induced injury.

56 Several transcription factors (TFs) can influence antioxidants and control OS. The nuclear factor
57 erythroid 2-related factor 2 (Nrf2) is a TF that controls several antioxidant genes, including heme
58 oxygenase 1 (HO-1) by binding to the antioxidant response elements (ARE) within the nucleus
59 and hence confers protection against OS and cell death (Satta et al., 2017, Hassanein et al., 2020).
60 In addition, sirtuin 1 (SIRT1) is the most extensively studied sirtuin that plays a crucial part in
61 histone deacetylation-mediated transcriptional regulation (Sauve et al., 2006). SIRT1 has a
62 significant redox regulator and essential for the antioxidant system (Singh et al., 2018). Also, it is
63 critical for DNA damage repair, apoptosis prevention, and OS resistance (Haigis and Guarente,
64 2006, Sayed et al., 2020). The O type superfamily of forkhead transcription factors known as
65 forkhead box O (FOXO) participates in many cell functions. FOXO3 is essential for cell survival
66 (Fasano et al., 2019) and can protect cells against OS (Reed et al., 2004). Several studies reported
67 that up-regulation of SIRT1/FOXO3 in lung tissue has a key role in mitigating oxidative lung
68 injury in several models (Zhang et al., 2015, Mahlooji et al., 2022).

69 Agomelatine (AGM), a melatonin analog, is a drug licensed for treating severe depression in
70 adults. It is a nonselective agonist for melatonin receptors 1 and 2 (MT1 and MT2) and has
71 antioxidant effects as revealed by several experimental models of disease (Yigitturk et al., 2017,
72 Eraslan et al., 2020, Cankara et al., 2021). Acute lung damage is reported to be improved by
73 melatonin (Zhang et al., 2016). In this study, we examined the protective role of AGM against Cd-
74 induced lung injury pointing to the involvement of OS and apoptosis, and the role of Nrf2/HO-1
75 and SIRT1/FOXO3 signaling.

76 **Materials and methods**

77 **Animals and treatments**

78 The present investigation included 32 male rats (*Rattus norvegicus*) weighing 190-210 g. These
79 animals were housed in standard cages (4 rats/cage) under a standardized environment ($22 \pm 1^\circ\text{C}$
80 and 50-60% humidity) with a 12 h light/dark cycle and had free access to water and food. The
81 experiment was approved by the research ethics Committee at Al-Azhar University (Approval no.:
82 AZ-AS/PHREC/26/2023).

83 The rats were randomly divided into four groups ($n=8$) based on the various treatments. The 1st
84 group (Control) received 0.5% carboxymethyl cellulose (CMC) orally for 14 days and the 2nd
85 group (AGM) received 25 mg/kg/day of AGM (Sigma, USA) suspended in 0.5% CMC orally for
86 14 days (Aguiar et al., 2013, Yucetas et al., 2019). Both the 1st and 2nd groups received a single
87 intraperitoneal (i.p.) injection of physiological saline on day 7. The 3rd group (Cd) received 0.5%
88 CMC orally for 14 days and a single i.p. injection of 1.2 mg/kg CdCl₂ (Sigma, USA) dissolved in
89 physiological saline on day 7 (de Lima et al., 2020). The 4th group (AGM/Cd) received 25 mg/kg

90 AGM orally for 14 days and a single i.p. injection of 1.2 mg/kg CdCl₂ on day 7. The dose volumes
91 for oral and i.p. administration were 5 ml/kg and 1 ml/kg body weight, respectively.

92 The dose and route of administration of Cd in this study were selected based on a pilot study and
93 previous investigations studied the toxicity of Cd (de Souza Predes et al., 2010, de Lima et al.,
94 2020, Handan et al., 2020). We have conducted a pilot study and the use of 1.2 mg/kg did not
95 cause mortality and resulted in blood Cd levels range between 1.36 and 1.75 µg/L which falls
96 within the ranges reported in human populations exposed to Cd. For instance, Mortada *et al* have
97 reported 0.8 – 4.5 µg/L blood Cd levels in an Egyptian population (Mortada et al., 2002).

98 The rats were anesthetized with ketamine (100 mg/kg i.p.) 24 h following the last treatment and
99 after scarification, the lungs were removed and separated into several parts. For further histological
100 and immunohistochemistry (IHC) examinations, one of these sections was collected on 10%
101 neutral buffered formalin (NBF), others on RNAlater and kept at -80°C, and the remaining part
102 was homogenized in Tris-HCl buffer (10% w/v), centrifuged, and the resultant supernatant was
103 stored at -80°C.

104 **Histopathology and immunohistochemistry (IHC)**

105 The lung tissue was fixed in 10% NBF for 24 h, then cleaned, embedded in paraffin, sectioned (4-
106 5 µm), and stained with H&E stain (Suvarna et al., 2013). Under the light microscope, the sections
107 were inspected blindly. Other paraffin sections were rehydrated with ethanol, dewaxed with
108 xylene, heated in citrate buffer, and then blocked with 1% BSA. The sections were incubated with
109 anti-Bax, anti-Caspase-3, and anti-Bcl-2 primary antibodies (Biospes, China) overnight at 4 °C
110 followed by washing and incubation with secondary antibody for 1 h. The color was developed
111 using DAB in H₂O₂ and the sections were counterstained with hematoxylin. A digital camera and

112 an Olympus light microscope were used to capture images and intensity of the resulting color was
113 evaluated using ImageJ.

114 **Biochemical assays**

115 ROS was determined by combining the samples with H₂DCF-DA (Sigma, USA) as previously
116 reported (Hozayen et al., 2019). Malondialdehyde (MDA) (Mihara and Uchiyama, 1978), reduced
117 glutathione (GSH) (Ellman, 1959) and activities of superoxide dismutase (SOD) (Marklund and
118 Marklund, 1974), glutathione-s-transferase (GST) (Keen et al., 1976), HO-1 (Abraham et al.,
119 1985), and GPx (Lawrence et al., 1976) were determined in the supernatant of the lung
120 homogenates. ELISA kit (ELabscience, China) was utilized to determine NADPH oxidase
121 following the provided instructions.

122 **qRT-PCR**

123 qRT-PCR was used to determine the changes in Nrf2, Keap1, glutamate-cysteine ligase catalytic
124 subunit (GCLC), HO-1, thioredoxin reductase 1 (TNXR1), NOX-2, SIRT1, and FOXO-3 mRNA
125 levels. Using Trizol reagent (Invitrogen), total RNA was extracted from lung tissue and
126 ThermoFisher Scientific reverse transcription kit was used to synthesize cDNA. SYBR Green
127 Master Mix and primers listed in Table 1 were used to amplify cDNA and the data were analyzed
128 using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001). The results were normalized to GAPDH.

129 **Statistical analysis**

130 The data are presented as mean \pm standard error of the mean (SEM). To compare the differences
131 between the groups, one-way ANOVA followed by Tukey's test were performed using GraphPad
132 Prism 8.0. A P value <0.05 was considered significant.

133 **Results**

134 **AGM mitigates lung histopathological alterations induced by Cd**

135 While control (Fig. 1A) and AGM-treated rats (Fig. 1B) showed normal histological structures,
136 significant histopathological alterations were recorded in the lung of Cd-treated rats (Fig. 1C-E &
137 Table 2). These included alveolar emphysema, interstitial hemorrhage, atelectasis, damage of the
138 bronchial epithelium, hyperplasia of peribronchial lymphoid tissue, smooth muscle fibers
139 fragmentation, and infiltration of the lamina propria with lymphocytes and interstitial connective
140 tissue (Fig. 1C-E). Pre-treatment with AGM remarkably ameliorated Cd-induced lung histological
141 abrasions with slight alveolar emphysema, foamy appearance, and hyperplasia of peribronchial
142 lymphoid tissue could be seen (Fig. 1F & Table 2).

143 **AGM attenuates Cd-induced pulmonary OS**

144 Cd significantly increased ROS (Fig. 2A) production and dramatically increased NOX-2 mRNA
145 (Fig. 2B) as well as NADPH-oxidase (Fig. 2C) in the lung compared to control group ($P < 0.001$).
146 The administration of Cd significantly increased lipid peroxidation marker MDA (Fig. 2D), and
147 depleted enzymatic and nonenzymatic antioxidants, including GSH (Fig. 3A), SOD (Fig. 3B), GPx
148 (Fig. 3C), and GST (Fig. 3D). AGM decreased ROS and MDA, downregulated NOX-2 mRNA
149 and NADPH-oxidase enzymatic activity and restored GSH, SOD, GPx, and GST in Cd-
150 administered rats.

151 **AGM attenuates Cd-induced apoptosis in the lung**

152 IHC we employed to study the impact of AGM on Cd-induced lung apoptosis (Fig. 4). The results
153 revealed that Cd significantly downregulated Bcl-2 (Fig. 4A,B) along with increased Bax (Fig.

154 4A,C) and caspase-3 (Fig. 4A,D) levels compared to control group ($P<0.001$). AGM treated group
155 showed significantly lower level of Bax and caspase-3 and higher levels of Bcl-2 protein.

156 **AGM modulates Keap1/Nrf2/HO-1 signaling in the lung of Cd-administered rats**

157 Cd markedly increased Keap1 mRNA (Fig. 5A), and decreased Nrf2 (Fig. 5B), GCLC (Fig. 5C),
158 HO-1 (Fig. 5D), and TNXRD1 (Fig. 5F) mRNA levels as well as HO-1 activity (Fig. 5E) in the
159 lung as compared with control group ($P<0.001$). AGM administration counterbalanced these
160 effects by increasing Nrf2, GCLC, HO-1 and TNXRD1 mRNA, as well as HO-1 activity while it
161 downregulated Keap1 mRNA significantly relative to that of Cd-intoxicated rats.

162 **AGM upregulates SIRT1 and FOXO3 in the lung of Cd-administered rats**

163 Cd significantly decreased SIRT1 (Fig. 6A) and FOXO3 (Fig. 6B) mRNA levels in the lung of
164 rats. In contrast. AGM potently upregulated SIRT1 and FOXO3 mRNA in the lung of Cd-
165 administered rats.

166 **Discussion**

167 Cadmium (Cd) is an extremely toxic heavy metal that has a detrimental effect on the body organs,
168 including the lung, as it can produce acute and chronic lung injury (Bernard, 2008, Rafati
169 Rahimzadeh et al., 2017). This study investigated the possible protective role of AGM against Cd-
170 induced lung toxicity. AGM attenuated Cd-induced lung injury by regulating Nrf2/HO-1 and
171 SIRT1/FOXO3 signaling and preventing OS and apoptosis.

172 Cd administration caused lung injury as shown by the tissue alterations such as damage to
173 bronchial epithelium and alveoli, infiltration of inflammatory cells, hemorrhage, emphysema, and
174 others. These findings showed the deleterious impact of Cd on the lung, effects that could be

175 explained by the surplus ROS generation which damage the cell macromolecules. The pathologies
176 of Cd toxicity have been linked to increased ROS and OS (Rashid et al., 2013). Cd does not
177 generate free radicals through redox reactions but generates H₂O₂ and NO and hydroxyl radicals
178 indirectly (Ikediobi et al., 2004, Cuypers et al., 2010). It can increase ROS generation via Fenton-
179 type mechanisms due to its ability to promote free iron release, leading to OS (Casalino et al.,
180 1997). OS is an imbalance between oxidants and antioxidants, leading to cellular disruption
181 mediated via oxidation of the cell macromolecules (Sies, 1997). Cellular antioxidants act as the
182 body's first line of defense against oxidative injury (Saha et al., 2016). OS in the lung of rats
183 received Cd was evidenced by elevated ROS and MDA, upregulated NOX2 and declined
184 antioxidants. NOXs are membrane-bound enzymes that function to generate ROS and NOX2
185 generates superoxide radicals as its product (Bedard and Krause, 2007) which can activate several
186 kinases, cytokines and TFs (Ma et al., 2017). The role of NOX-mediated ROS production in Cd
187 toxicity and vascular damage was reported (Pinheiro Júnior et al., 2020) and its role in pulmonary
188 fibrosis and airway and pulmonary diseases has been previously reviewed (Lee and Yang, 2012,
189 Kato and Hecker, 2020). Owing to the role of OS in Cd toxicity, attenuation of excess ROS would
190 be effective in protecting the lung against injury.

191 AGM attenuated lung injury, decreased ROS and MDA, downregulated NADPH oxidase and
192 boosted antioxidants in the lung of Cd-administered rats. Thus, it is noteworthy assuming that the
193 protective effect of AGM was mediated via attenuation of OS. In accordance, studies have shown
194 that the role of AGM in protecting the lung against methotrexate toxicity has been associated with
195 suppressed OS (Kamel et al., 2022). AGM prevented histopathological alterations induced by
196 lipopolysaccharide (LPS) in the lung of rats by suppressing cytokines (Köse et al., 2021). The
197 antioxidant efficacy of AGM is supported by studies involving disease models of nephro- and

198 neurotoxicity (Cankara et al., 2021, Mahmoud et al., 2021). Similar to melatonin, AGM can
199 protect the cells against injury by scavenging ROS and maintaining redox homeostasis through its
200 binding with melatonergic receptors (Paulis et al., 2012, Galano and Reiter, 2018). This protective
201 role was supported by the investigation of apoptosis markers that showed the ability of AGM to
202 prevent apoptotic cell death. Besides the surplus ROS, Cd upregulated pro-apoptotic proteins and
203 decreased Bcl-2 in the lung of rats demonstrating cell death via apoptosis. ROS activate Bax and
204 both can deteriorate mitochondrial membrane potential (MMP), resulting in the outflow of
205 cytochrome c into the cytosol to activate caspase-3 (Shi et al., 2003). Cleavage of DNA and
206 structural and cell cycle proteins by activated caspase-3 lead to cell death (Thomsen et al., 2013).
207 Hence, suppression of surplus ROS by AGM mediated its protective role against Cd-induced
208 apoptosis in the lung of rats.

209 To further explore the beneficial role of AGM against Cd-induced lung injury, we determined its
210 effect on Nrf2/HO-1 signaling, SIRT1 and FOXO3. Nrf2 is a TF widely expressed in several
211 organs and tissues, including the lung. The Keap1 repressor protein controls Nrf2 in the cytoplasm
212 through the ubiquitin-proteasome pathway degradation (Furukawa and Xiong, 2005). In response
213 to elevated ROS, Nrf2 dissociates from Keap1, relocates to the nucleus, and transactivates a
214 number of antioxidants, including HO-1 that exerts antioxidant and anti-inflammatory activities
215 (Satta et al., 2017). HO-1 catalyzes the degradation of heme into the radical scavenger bilirubin
216 (Siow et al., 1999), resulting in attenuation of OS and confers protection against cell death.
217 TXNRD1 plays a critical role in redox homeostasis by reducing and activating the oxidoreductase
218 thioredoxin. The latter contains a dithiol-disulfide active site and thereby can reduce the oxidized
219 cysteine residues on proteins subjected to oxidation by ROS. It binds ROS and therefore prevent
220 these oxidizing species from harming the cells via OS and can also inhibit apoptosis signal

221 regulating kinase 1 (Cadenas et al., 2010). Nrf2 also increases the transcription of GCLC, a rate-
222 limiting enzyme in the biosynthesis of GSH that protects against OS (Lu, 2013). Cd markedly
223 upregulated gene expression of Keap1, and decreased Nrf2, GCLC, HO-1, and TNXRD1. Cd is
224 known to downregulate Nrf2/HO-1 signaling and decrease the antioxidant levels in different
225 organs (Alam et al., 2021, Bakr et al., 2022). Interestingly, AGM counteracted these effects and
226 enhanced Nrf2, GCLC, HO-1, and TNXRD1, along with the downregulation of Keap1.
227 Upregulation of Nrf2 signaling coincided with the suppressed ROS and OS and increased GSH
228 and antioxidant enzymes. These findings pinpointed the role of Nrf2/HO-1 and Nrf2/TNXRD1
229 pathways in mediating the antioxidant activity of AGM.

230 Moreover, through the deacetylation of substrate proteins under OS, SIRT1 significantly
231 safeguards the cell and promotes cell survival (Guarente, 2013, Sayed et al., 2020). SIRT1 targets
232 FOXOs which have a role in many physiological and cellular processes, including cell
233 proliferation, apoptosis, and response to ROS (Tzivion et al., 2011). FOXOs play a critical role in
234 cell survival by transactivating antioxidant enzymes like SOD (Tzivion et al., 2011). SIRT1
235 inhibits the transcription of genes involved in apoptosis while increasing the expression of FOXO
236 target genes involved in stress tolerance (Greer and Brunet, 2005). It deacetylates PPAR γ
237 coactivator 1 alpha that promotes the transcription of SOD and catalase (Olmos et al., 2013), and
238 p53 resulting in inhibiting its OS-induced apoptotic cell death (Vaziri et al., 2001). Our results
239 indicated that Cd downregulated SIRT1 and FOXO3, effects that were prevented by AGM. We
240 have previously showed the ability of AGM to upregulate SIRT1 and prevent gentamicin
241 nephrotoxicity in rats (Mahmoud et al., 2021). Thus, upregulation of SIRT1 contributed to AGM-
242 mediated suppression of OS and apoptosis provoked by Cd. However, the lack of protein
243 quantification of SIRT1 and FOXO3 could be considered a limitation of this study.

244 **Conclusion**

245 Our study provides new information on the protective effect of AGM against Cd-induced lung
246 injury. AGM mitigated tissue injury, downregulated NADPH oxidase and ROS generation,
247 decreased lipid peroxidation, and prevented apoptosis. These effects were linked to enhanced Nrf2,
248 GCLC, HO-1, TNXRD1, and SIRT1. Taken together, AGM has a protective effect against Cd-
249 induced lung injury via its antioxidant and anti-apoptotic effects. It is effective in conferring
250 protection against Cd lung intoxication, pending further investigations to explore other
251 mechanism(s) of action.

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255 **Data availability**

256 The authors confirm that the data supporting the findings of this study are available within the
257 article.

258 **Disclosure statement**

259 The authors declare that no conflict of interest exists.

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420

421

422 **Tables:**

423 Table 1. Primers used for qRT-PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Keap1</i>	TCAGCTAGAGGCGTACTGGA	TTCGGTTACCATCCTGCGAG
<i>Nrf2</i>	ATTGCTGTCCATCTCTGTCAG	GCTATTTTCCATTCCCGAGTTAC
<i>HO-1</i>	TGCTTGTTTCGCTCTATCTCC	CTTTCAGAAGGGTCAGGTGTC
<i>GCLC</i>	GTTGTTACTGAATGGCGGCG	CGGCGTTTCCTCATGTTGTC
<i>TXNRD-1</i>	TTGGAGTATGGCTGCTGTGG	TGCAGCCTTCTTCTACCTGC
<i>NOX-2</i>	CTGGGCTGTGAATGAGGGAC	TGTGAATGGCCGTGTGAAGT
<i>SIRT1</i>	CGGTCTGTCAGCATCATCTTCC	CGCCTTATCCTCTAGTTCCTGTG
<i>FOXO3</i>	GCCTCATCTCAAAGCTGGGT	AGTTCTGCTCCACGGGAAAG
<i>GAPDH</i>	TGCTGGTGCTGAGTATGTCG	TTGAGAGCAATGCCAGCC

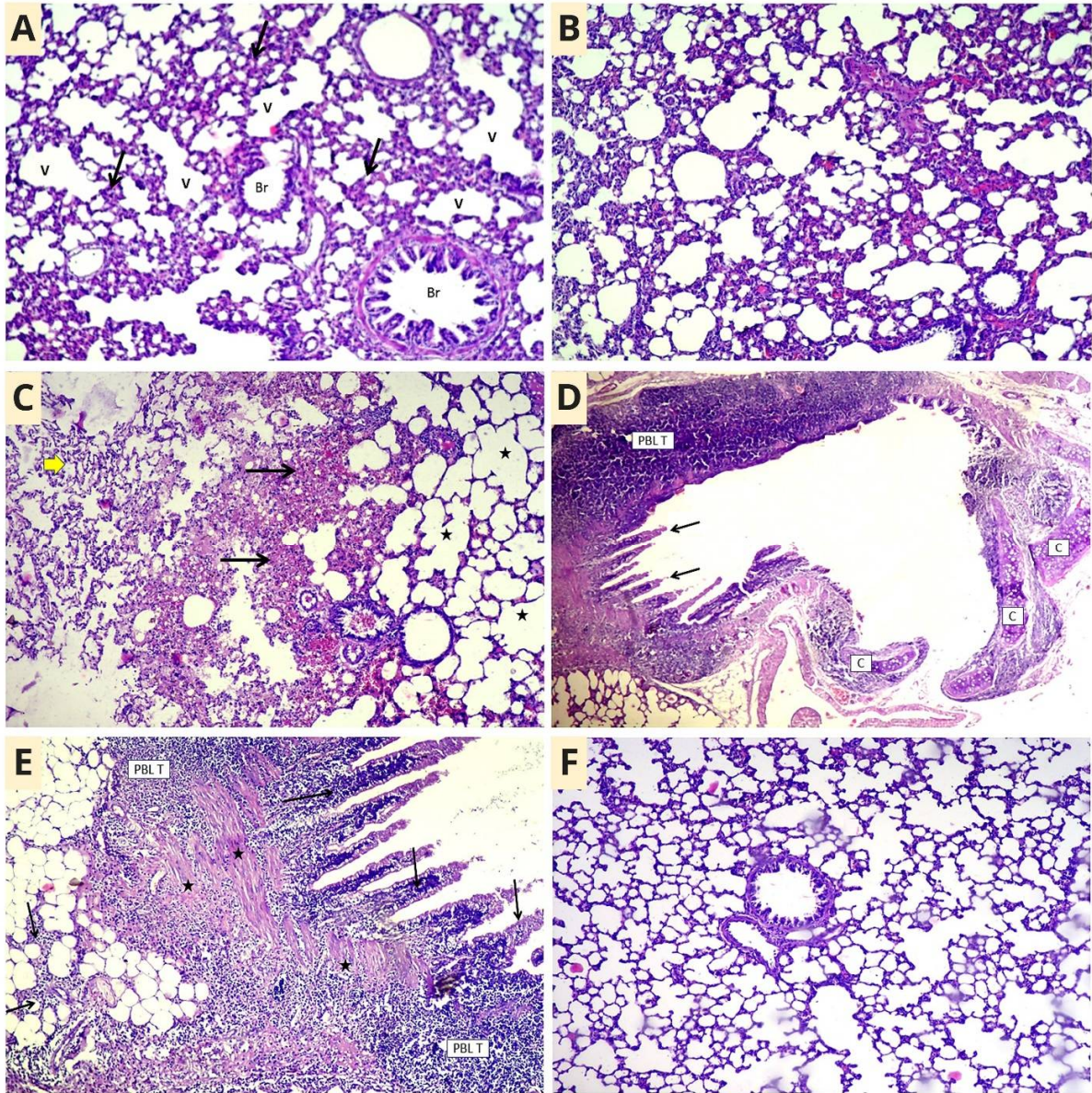
424

425

426 Table 2. Scoring of the histopathological lesions.

	Control	AGM	Cd	AGM/Cd
Alveolar emphysema	-	-	+++	++
Interstitial hemorrhage	-	-	+++	-
Atelectasis	-	-	++	-
Damage of the bronchial epithelium	-	-	+++	-
Hyperplasia of peribronchial lymphoid tissue	-	-	+++	+
Infiltration of the lamina propria with lymphocytes and interstitial connective tissue	-	-	++	-
Smooth muscle fibers fragmentation	-	-	++	-

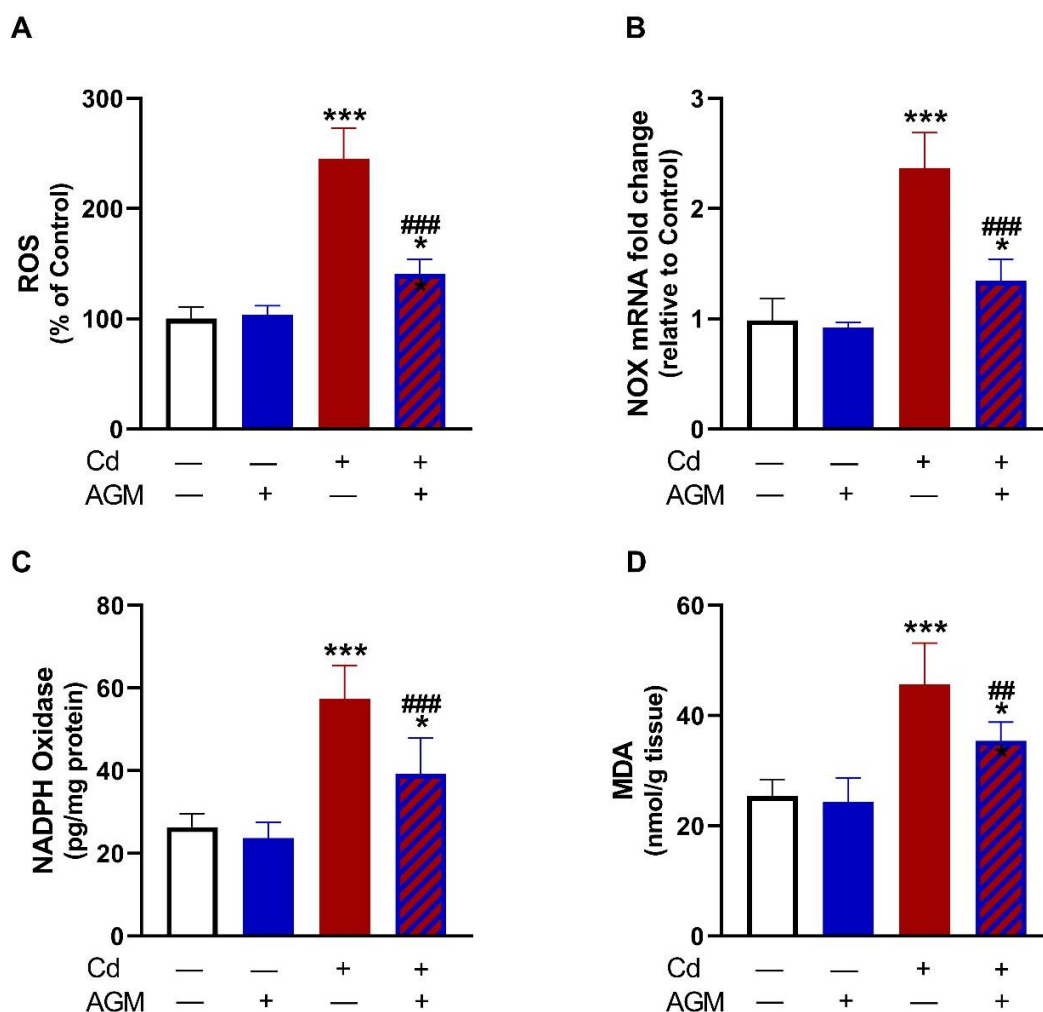
427



429

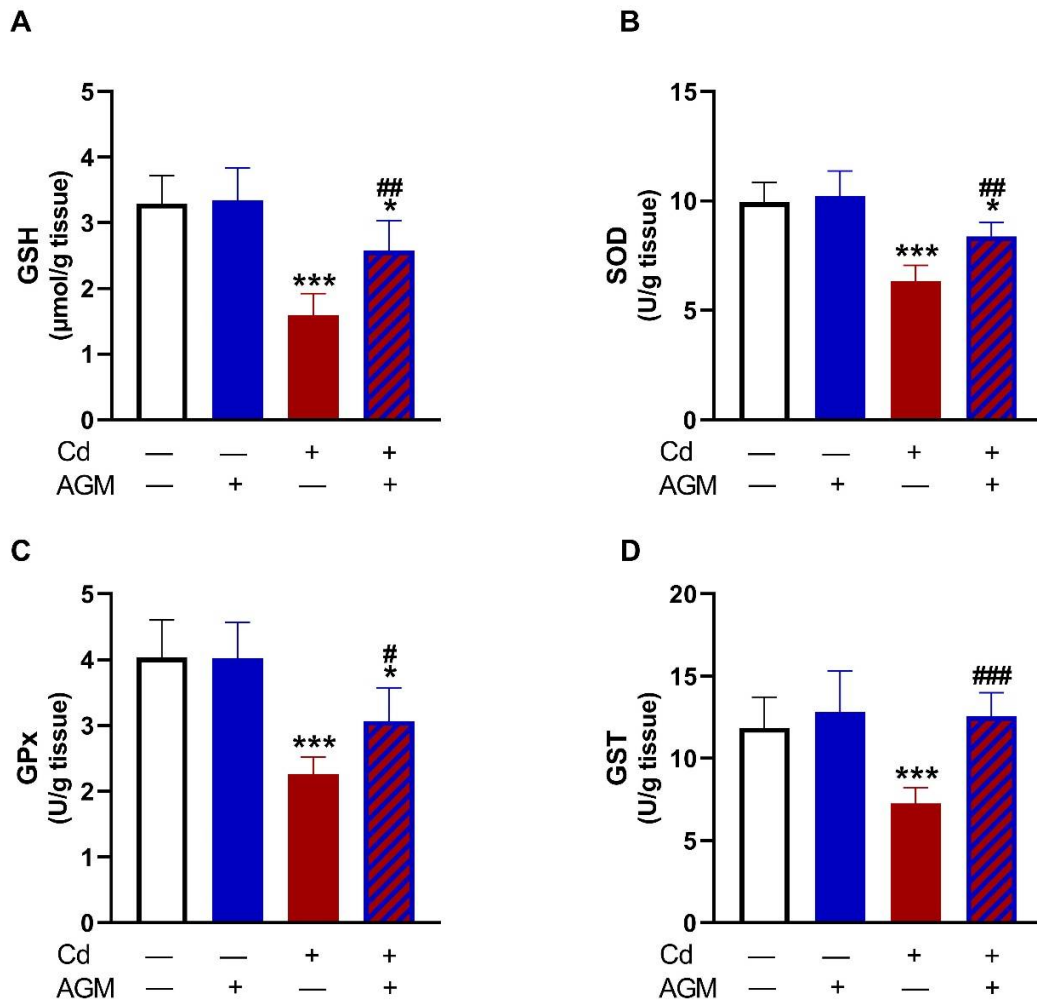
430 Fig. 1. AGM mitigated lung histopathological alterations induced by Cd. H&E-stained sections in
431 lung of (A) Control and (B) AGM-supplemented rats showing the normal appearance of alveoli
432 (v), interstitial tissue (arrow) and bronchioles (Br), (C-E) The lungs of Cd-intoxicated rats showed
433 alveolar emphysema (star), interstitial hemorrhage (black arrow), slight atelectasis (thick yellow
434 arrow) [C], damage of bronchial epithelium (arrow) and hyperplasia of peribronchial lymphoid

435 tissue (PBLT) [D], damage of bronchial epithelium and infiltration of the lamina propria with
 436 lymphocytes, interstitial connective tissue (arrow), hyperplasia of PBLT and smooth muscle fibers
 437 fragmentation (star) [E], and (F) Cd-administered rats treated with AGM showing noticeable
 438 improvement of the emphysema with an absence of interstitial hemorrhage. (Scale bar = 200 μ m).



439
 440 Fig. 2. AGM decreased ROS (A), NOX-2 mRNA (B), NADPH-oxidase (C) and MDA (D) in the
 441 lung of Cd-administered rats. Data are Mean \pm SEM, ($n = 8$). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs
 442 Control. ## $P < 0.01$ and ### $P < 0.001$ vs Cd.

443

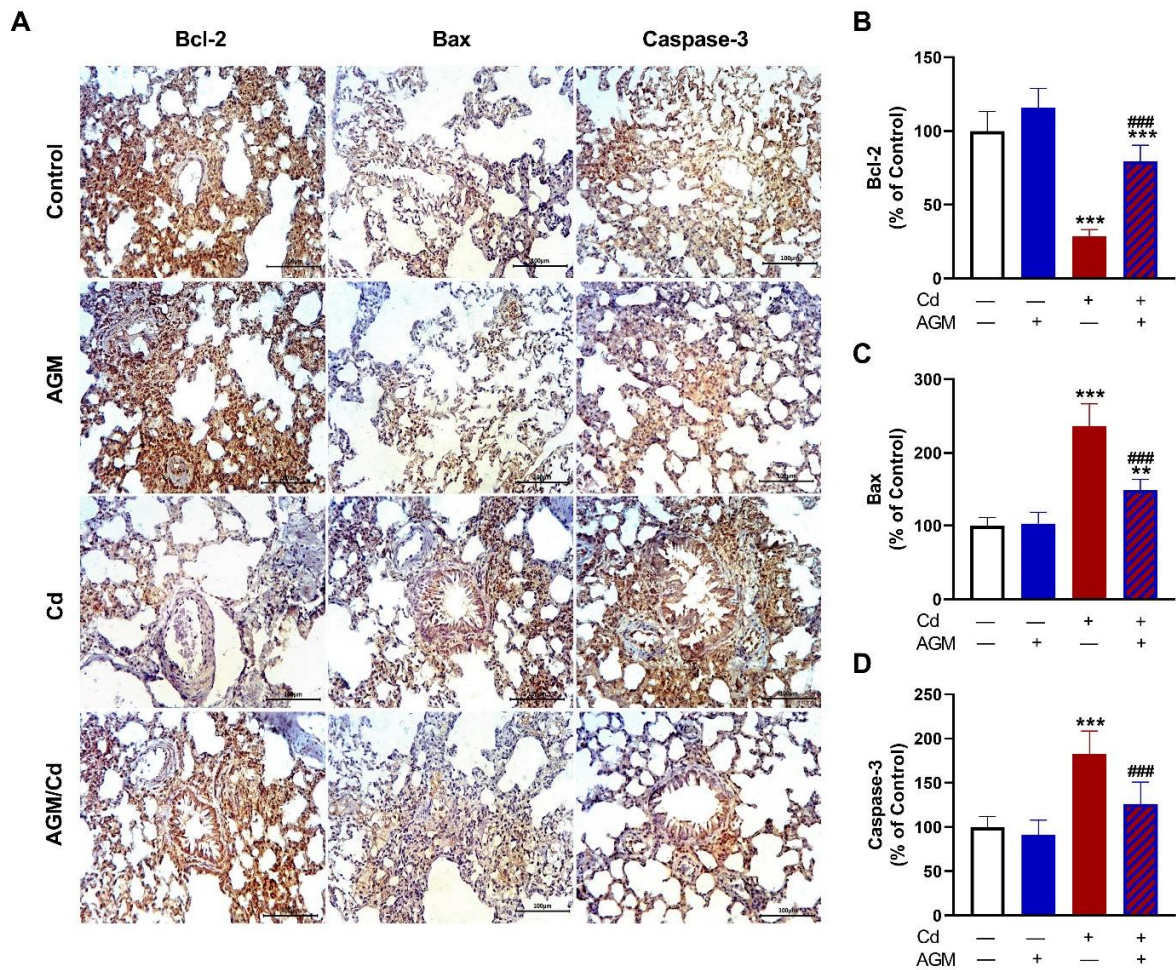


444

445 Fig. 3. AGM increased GSH (A), SOD (B), GPx (C) and GST (D) in the lung of Cd-administered

446 rats. Data are Mean \pm SEM, ($n = 8$). * $P < 0.05$ and *** $P < 0.001$ vs Control. # $P < 0.05$, ## $P < 0.01$ and

447 ### $P < 0.001$ vs Cd.



448

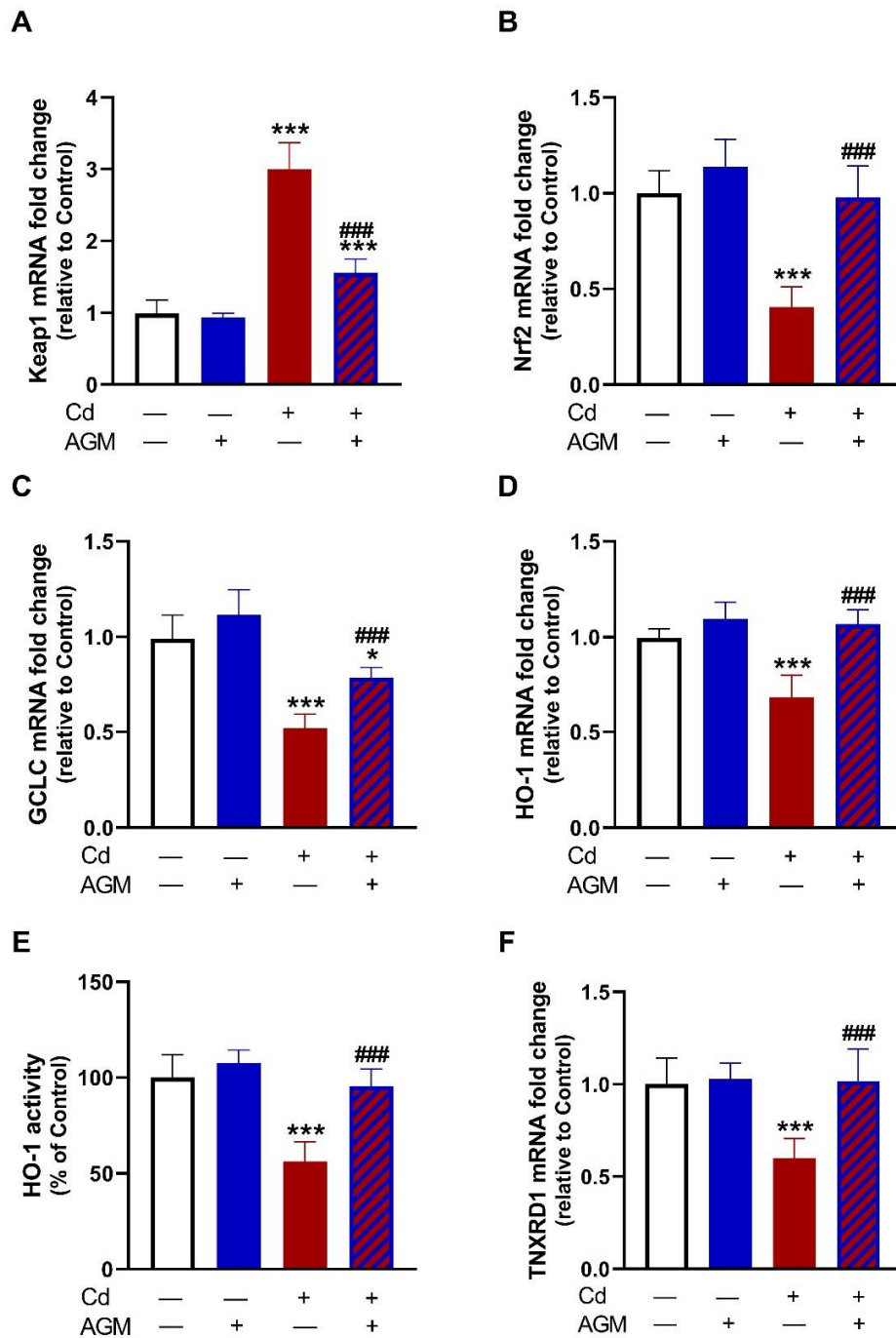
449 Fig. 4. AGM attenuated Cd-induced apoptosis in the lung of rats. (A) Immunostaining of Bcl-2,

450 Bax and caspase-3 in the lung of control and treated rats. (B-D) Image analysis showing decreased

451 Bcl-2 (B) and increased Bax (C) and caspase-3 (D) in the lung of Cd-administered rats and the

452 protective role of AGM. Data are Mean \pm SEM, ($n = 8$). ** $P < 0.01$ and *** $P < 0.001$ vs Control.

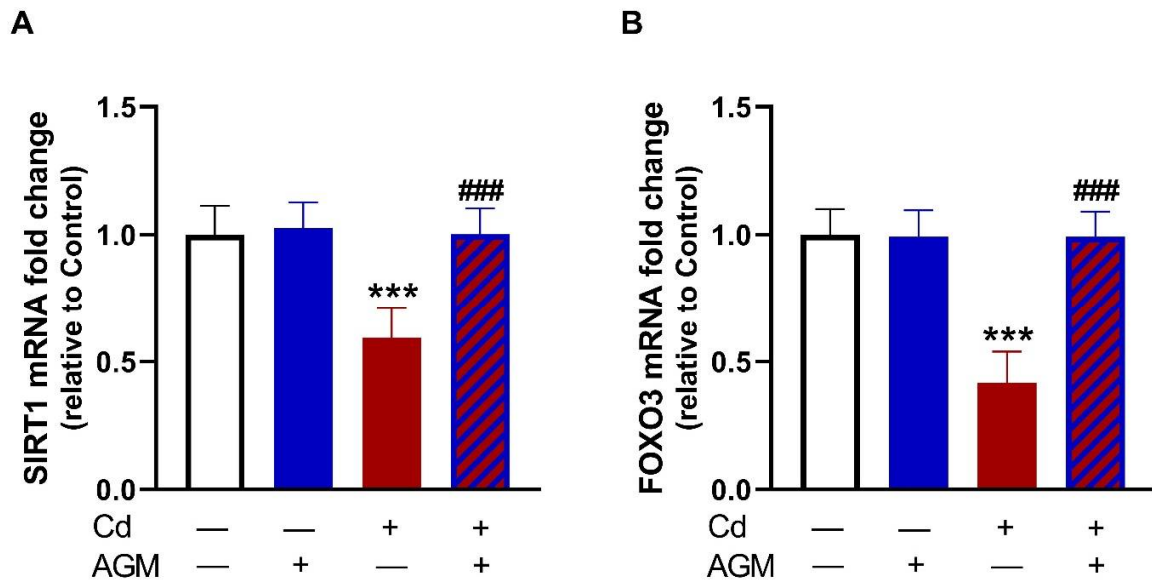
453 ### $P < 0.001$ vs Cd.



454

455 Fig. 5. AGM upregulated Nrf2 signaling in the lung of Cd-administered rats. AGM decreased
 456 Keap1 mRNA (A), and increased Nrf2 (B), GCLC (C), HO-1 (D) and TNFRD1 (F) mRNA and

457 HO-1 activity (E) in Cd-administered rats. Data are Mean \pm SEM, ($n = 8$). * $P < 0.05$ and *** $P < 0.001$
458 vs Control. ### $P < 0.001$ vs Cd.



459

460 Fig. 6. AGM upregulated SIRT1 (A) and FOXO3 (F) in the lung of Cd-administered rats. Data are
461 Mean \pm SEM, ($n = 8$). *** $P < 0.001$ vs Control and ### $P < 0.001$ vs Cd.

462