ISSN: 2458-8989

Natural and Engineering Sciences

NESciences, 2026, 11 (3): 676-688 doi: 10.28978/nesciences.263054

Organ-Specific Morphological and Biochemical Responses to Chronic Carbon Monoxide Exposure and Their Modulation by Plant-Derived Phytocorrection

Teshaev Shukhrat Jumayevich[1*], Hamroyev Izzat Sayfilloyevich[2], Muhammadjon Axmadjon o‘g‘li Raxmonberdiyev[3], Kurbanova Nodira Isomitdinovna[4], Akhmedova Sayyora Muhamadovna[5]

[1]Doctor of Medical Sciences, Professor of the Department of Anatomy, Clinical Anatomy and Operative Surgery with Topographic Anatomy at the Bukhara State Medical Institute named after Abu Ali ibn Sino. Bukhara, Uzbekistan. ORCID ID: https://orcid.org/0009-0002-1996-4275, E-mail: Teshayev@Bsmi.Uz

[2]Republican Scientific Center for Emergency Medical Care, Bukhara Branch. ORCID: https://orcid.org/0000-0002-3898-509, E-mail: izzathamroyev1gmail.com

[3]Senior Lecturer, Department of Anatomy, Operative Surgery and Topographic Anatomy, Normal Physiology, Forensic Medicine and Pathology, Chirchiq Branch of Tashkent State Medical University, Tashkent, Uzbekistan. ORCID ID: https://orcid.org/0009-0000-1212-7226, E-mail: mrahmonberdiev466@gmail.com

[4]PhD, Associate Professor of the department of therapeutic dentistry of the Bukhara State Medical Institute named after Abu Ali ibn Sino., ORCID: https://orcid.org/0009-0000-4280-3205, E-mail: nodira.isomiddinovna.78@gmail.com, kurbanova.nodira@bsmi.uz

[5]Doctor of Medical Sciences (DSc), Professor, Head of the Department of Anatomy and OSTA, Tashkent State Medical University, Uzbekistan. ORCID: https://orcid.org/0009-0001-1881-4710 , E-mail: Sayyora-76@mail.ru

Abstract

Background. Chronic exposure to carbon monoxide (CO) may induce persistent tissue hypoxia, oxidative and metabolic disturbances, microcirculatory impairment, and structural remodeling in organs with different functional characteristics. Although the morphological consequences of CO exposure have been investigated in individual organs, comparative assessment of hypoxia-associated remodeling across the lung, kidney, and prostate remains limited.

Objective. To comparatively characterize morphological and morphometric alterations in the lung, kidney, and prostate of rats following chronic CO exposure and to evaluate the structural effects of phytocorrection using Gulimahsar infusion and Asparagus officinalis oil.

Materials and Methods. Male white rats of different ages were exposed to CO under experimental conditions for one month. Morphological changes were assessed using histological and histochemical methods, while quantitative evaluation was performed using digital morphometric analysis. Immunohistochemical assessment included hypoxia-associated and proliferative markers, with particular attention to HIF-1α in the kidney and prostate and Ki-67 in the lung. Digital histological

Corresponding Author: Teshaev Shukhrat Jumayevich, E-mail: Teshayev@Bsmi.Uz

images were analyzed using QuPath-based morphometric approaches. Correction groups received plant-derived preparations during the experimental period.
Results. Chronic CO exposure was associated with organ-specific structural remodeling. In the lung, exposure produced alveolar enlargement, septal alterations, emphysematous changes, and reduction of capillary density, with more pronounced changes in older animals. In the kidney and prostate, chronic CO exposure was associated with dystrophic, inflammatory, and stromal-parenchymal alterations. HIF-1α immunoreactivity increased in the kidney and prostate following CO exposure, indicating activation of a tissue hypoxia-associated response. Phytocorrection was accompanied by partial restoration of tissue architecture, reduction of emphysematous changes in the lung, improvement of renal and prostatic structural parameters, and normalization of immunohistochemical responses.
Conclusion. Chronic CO exposure produces a systemic pattern of hypoxia-associated morphological remodeling with distinct organ-specific manifestations in the lung, kidney, and prostate. The observed structural improvement following phytocorrection suggests that plant-derived interventions may attenuate CO-associated tissue injury. Integrated morphometric and immunohistochemical assessment may provide a useful approach for characterizing chronic hypoxic tissue injury.

Keywords
Carbon monoxide; chronic exposure; hypoxia; HIF-1α; lung; kidney; prostate; morphometry; immunohistochemistry; phytocorrection; digital pathology.

Available Online: 10/09/2026

Introduction

Carbon monoxide (CO) is a toxic environmental and occupational pollutant capable of producing systemic biological effects through interference with oxygen transport and tissue oxygen utilization. The biological consequences of CO exposure are not restricted to acute poisoning (Chenoweth *et al*., 2021). Persistent exposure may be associated with prolonged hypoxic stress, oxidative imbalance, inflammatory responses, mitochondrial dysfunction, and subsequent structural alterations in target tissues (Rose *et al*., 2017).
The development of tissue hypoxia represents an important mechanism underlying the morphological consequences of chronic CO exposure (Iciek *et al*., 2022). Carbon monoxide binds to hemoglobin and contributes to impaired oxygen delivery, while its effects on cellular metabolism may additionally influence mitochondrial function and oxidative homeostasis (Bae *et al*., 2024). Hypoxia-responsive signaling is therefore of particular interest when evaluating chronic CO-associated tissue injury (Duan, 2016; Ahmadi, 2025). The hypoxia-inducible factor system, particularly HIF-1α, represents an important component of cellular adaptation to reduced oxygen availability (Muto, 2017; Hassan *et al*., 2025).
The kidney is particularly relevant for the investigation of hypoxia-associated injury as its structural organization and high metabolic requirements create regional differences in oxygen availability (Huang *et al*.,2019). Previous studies cited in the dissertation indicate that chronic hypoxia may contribute to tubular and interstitial injury, fibrosis, and alterations in renal tissue homeostasis (Sugahara *et al*., 2020). HIF signaling has consequently been investigated as an important component of the renal response to hypoxic stress (Wu *et al*., 2025)
The prostate represents another potentially important target of chronic environmental stress. Its stromal and epithelial components are closely interconnected, and changes in the local microenvironment, oxidative balance, and oxygen availability may influence tissue remodeling (Ow *et al*., 2018). The source dissertation specifically identifies the need for integrated morphological, morphometric, and immunohistochemical evaluation of the prostate under chronic CO exposure (Cavadas *et al*., 2017).
The lung requires a different interpretation as it represents the principal organ directly responsible for gas exchange. Chronic CO exposure was associated with hypoxic-destructive and emphysematous remodeling, alterations of alveolar dimensions, changes in alveolar septa, and impairment of the microcirculatory component (Pugh and Ratcliffe, 2017). These changes became more pronounced with increasing animal age.
An important feature of the lung findings was the quantitative nature of the observed remodeling. In 3-month-old CO-exposed animals, the mean alveolar area reached 4018±146 μm², compared with the lower values observed in the corresponding control animals. In 9-month-old exposed animals, the

mean alveolar area increased further to 5286±184 μm², while emphysematous areas reached 26.9±1.1% (Ganjavi *et al.*, 2022).

The structural changes observed in the lung were accompanied by alterations in proliferative activity. Ki-67 immunohistochemical assessment demonstrated increased proliferative activity following chronic CO exposure, consistent with a compensatory-reparative response to hypoxic and destructive tissue changes (Vickman *et al.*, 2020). Digital analysis was performed using QuPath, with DAB-positive nuclei identified and quantitatively evaluated.

Phytocorrection represents a potentially relevant approach for attenuating chronic toxic and hypoxic tissue injury. In the lung study, correction with Gulimahsar infusion was associated with a reduction in emphysematous remodeling. In 9-month-old animals, the mean alveolar area decreased from 5286±184 to 4026±152 μm², while the emphysematous area decreased from 26.9±1.1% to 12.9±0.5%. At the same time, alveolar number and capillary density increased (Okada, 2016).

Chronic CO exposure was modeled using an inhalational concentration of 0.01–0.05 mg/L for one month. Correction groups received either Asparagus officinalis oil at 0.1 mL/animal/day or Gulimahsar infusion prepared at a 1:10 ratio and administered at 0.1 mL/animal/day for 30 days. Histological, histochemical, morphometric, and immunohistochemical analyses were subsequently performed.

Digital pathology provided an additional quantitative component of the investigation. Kidney and prostate preparations were analyzed using digital images, with QuPath 0.5.0 used for morphometric assessment, including tissue segmentation, area measurements, and quantitative evaluation of structural parameters (Levesque and Nelson, 2018). At least 10 randomly selected microscopic fields were analyzed for each preparation.

The HIF-1α findings provide a mechanistic component linking morphological remodeling with tissue hypoxia (Bonkhoff, 2018; Mateshuk-Vatseba *et al*., 2023). In the kidney, chronic CO exposure increased the proportion of HIF-1α-positive cells, while correction was associated with a reduction in this response. In the prostate, HIF-1α expression was also increased following CO exposure, reaching particularly high levels in the 9-month experimental group, where 70.27% of analyzed cells were HIF-1α positive (Mohamed *et al*., 2023; Ali *et al*., 2023). These findings support the presence of an organ-specific hypoxia-associated response to chronic CO exposure (McKenna *et al.*, 2018; Bankhead *et al*., 2017).

Despite these findings, most previous experimental approaches have considered individual organs separately. A comparative framework encompassing the lung, kidney, and prostate may therefore provide additional information concerning the common hypoxia-associated mechanisms and the organ-specific morphological manifestations of chronic CO exposure (Krmpotic *et al*., 2024).

Accordingly, the aim of the study was to comparatively characterize the morphological and morphometric changes induced by chronic CO exposure in the lung, kidney, and prostate of rats, with particular emphasis on hypoxia-associated tissue remodeling and HIF-1α expression, and to evaluate the morphological effects of phytocorrection.

Materials and Methods

2.1. Experimental design and laboratory animals

The experimental study was performed on male white laboratory rats. At the beginning of the experiment, the animals were 2 and 8 months of age. The one-month experimental period was taken into account when determining the age of the animals at the time of morphological examination; therefore, the animals were 3 and 9 months of age, respectively, at the end of the experiment.

A total of 250 animals were initially included in the experimental design. The animals were distributed among control, chronic carbon monoxide (CO)-exposed, and correction groups. During the experimental period, 10 animals died, after which the final morphological, morphometric, immunohistochemical, and statistical analyses were performed on 240 animals.

Table 1. Experimental design and distribution of animals

Group	Experimental condition	Initial age	Age after 1-month exposure	Number of animals initially
I	Control	2 months	3 months	60
I	Control	8 months	9 months	60
II	Chronic CO exposure	2 months	3 months	35*
II	Chronic CO exposure	8 months	9 months	35*

III	CO + *Asparagus officinalis* L. oil	2 months	3 months	30
III	CO + *Asparagus officinalis* L. oil	8 months	9 months	30
IV	CO + Gulimahsar infusion	2 months	3 months	30
IV	CO + Gulimahsar infusion	8 months	9 months	30

*The chronic CO-exposure group comprised 70 animals in total. Ten animals died during the experimental period; therefore, 240 animals were included in the final analysis.

Before the beginning of the experiments, the animals underwent a quarantine period and were subsequently maintained under standard vivarium conditions. Throughout the study, the general condition and behavior of the animals were monitored.

2.2. Experimental groups and chronic carbon monoxide exposure

To reproduce chronic CO-associated hypoxic stress, experimental animals were exposed to carbon monoxide by inhalation for 1 month. The concentration of CO in the inhaled air was 0.01–0.05 mg/L. The experimental design included:

Group I — Control: animals maintained under standard conditions;

Group II — CO exposure: animals subjected to chronic CO exposure for 1 month;

Group III — CO + Asparagus officinalis L. oil: animals exposed to CO and simultaneously receiving phytocorrection with Asparagus officinalis L. oil;

Group IV — CO + Gulimahsar infusion: animals exposed to CO and simultaneously receiving Gulimahsar plant infusion.

The original experimental design included 60 animals in the control group, 70 animals in the chronic CO group, 60 animals in the Asparagus officinalis L. oil correction group, and 60 animals in the Gulimahsar infusion correction group. 10 animals died during the experimental period, the final analyses were conducted on 240 animals.

Importantly, the age of the animals is reported according to the stage of the experiment: 2 and 8 months at enrollment and 3 and 9 months, respectively, after the one-month experimental period. Thus, these age designations represent different time points of the same experimental protocol rather than different experimental cohorts.

2.3. Phytocorrection

Two plant-derived preparations were investigated for their potential corrective effects against chronic CO-associated morphological alterations. Asparagus officinalis L. oil (Qushqonmas oil) was administered intragastrically at a dose of 0.1 mL/animal/day during the correction period. Gulimahsar infusion was administered intragastrically at a dose of 0.1 mL/animal/day. The infusion was prepared from dried and ground plant material at a 1:10 ratio. According to the experimental rotocol, the plant material was extracted with hot water, followed by filtration and cooling to room temperature before administration. The correction period lasted 1 month and corresponded to the chronic CO exposure period. For the pulmonary component of the study, the dissertation also describes intragastric administration of 0.1 mL of Asparagus officinalis oil and 0.1 mL of Gulimahsar infusion. The Gulimahsar preparation was administered orally twice daily during the correction period.

2.4. Tissue collection and histological processing

At the end of the experimental period, tissue samples from the lungs, kidneys, and prostate glands were collected for morphological and morphometric examination.

For renal and prostatic tissues, specimens were fixed in 10% neutral buffered formalin for 24–48 h. The samples were subsequently dehydrated through ascending concentrations of ethanol (70%, 80%, 90%, 96%, and absolute ethanol), cleared in xylene, and embedded in paraffin.

Paraffin blocks were sectioned at a thickness of 5–7 μm using a Leica RM2235 semi-automatic microtome (Leica Biosystems, Germany). The sections were transferred to glass slides and dried before staining.

Routine histological examination was performed using hematoxylin and eosin (H&E) staining. Van Gieson staining was used to assess collagen and fibrotic tissue changes, while Alcian blue staining was used for the assessment of acidic mucopolysaccharides where appropriate.

For pulmonary tissue, H&E and Van Gieson staining were used to evaluate the alveolar apparatus, bronchial structures, interstitial tissue, and vascular components.

2.5. Pulmonary morphometry

Morphometric evaluation of the lung was performed to characterize structural changes associated with chronic CO exposure.The following parameters were assessed: alveolar area; alveolar diameter; alveolar wall/septal thickness; relative alveolar volume; number of alveoli; bronchial wall thickness; vascular and capillary density; relative area of emphysematous changes; collagen/connective-tissue changes.These parameters were compared between control animals, CO-exposed animals, and animals receiving phytocorrection.

2.6. Renal morphometry

Renal morphological and morphometric assessment included evaluation of the cortical region and nephron structures.

The following parameters were measured: glomerular diameter; glomerular area; width of the Bowman–Shumlyansky capsule space; outer and inner diameters of proximal and distal tubules; tubular epithelial height; tubular luminal area; interstitial tissue area; glomerular density; relative proportion of fibrotic tissue. These parameters were used to quantify glomerular, tubular, and interstitial remodeling associated with chronic CO exposure.

2.7. Prostatic morphometry

Morphometric examination of the prostate focused on the secretory acinar apparatus and stromal-parenchymal relationships. The principal parameters included: acinar diameter; secretory epithelial height; relative proportion of stromal tissue; relative proportion of parenchymal tissue; structural characteristics of the acinar-secretory apparatus. Morphometric measurements were performed on representative histological fields from control, CO-exposed, and phytocorrected animals.

2.8. Immunohistochemistry

Immunohistochemical examination was used to characterize cellular responses associated with chronic hypoxic stress and tissue remodeling. For the lung, Ki-67 expression was assessed as an indicator of proliferative activity. Immunohistochemical staining was performed using a DAB chromogen, and the stained sections were examined microscopically. Pulmonary Ki-67 preparations were digitally analyzed using QuPath 0.4.0.For the kidney and prostate, HIF-1α expression was evaluated as a marker associated with the cellular response to hypoxic conditions. The percentage of immunopositive cells and, where available, staining intensity and positive-cell density were quantified. The comparative evaluation of HIF-1α expression in the kidney and prostate enabled assessment of the organ-specific hypoxic response to the same chronic CO exposure.

2.9. Digital pathology and morphometric analysis

Digital images of histological and immunohistochemical preparations were analyzed using QuPath software. For pulmonary Ki-67 analysis, QuPath 0.4.0 was used. For renal and prostatic histological and immunohistochemical analysis, QuPath 0.5.0 was used. Digital image analysis included segmentation and identification of tissue structures, measurement of areas and linear dimensions, quantification of cellular and tissue components, and assessment of immunohistochemical marker expression.

For HIF-1α, digital analysis included determination of: total number of analyzed cells; number of HIF-1α-positive cells; percentage of positive cells; positive-cell density; staining intensity, where available. For Ki-67, the proportion of immunopositive cells was quantified.

Digital morphometry was used to improve the objectivity and reproducibility of measurements and to facilitate direct comparison between experimental groups.

2.10. Statistical analysis

The experimental data were processed using Microsoft Excel 2016 and IBM SPSS Statistics version 26.0. Quantitative variables were summarized using descriptive statistical parameters. Differences between experimental groups were evaluated using the statistical approaches applied in the original dissertation datasets. Statistical significance was considered at $P < 0.05$.

The statistical analysis was used to evaluate: age-related morphological differences; the effect of chronic CO exposure; differences between lung, kidney, and prostate responses; changes following phytocorrection; quantitative changes in Ki-67 and HIF-1α expression.

2.11. Integrated comparative analysis

The three target organs were analyzed within a unified morphological framework.

The lung was considered a primary respiratory target in which chronic CO exposure could directly affect gas-exchange structures and the alveolar-capillary interface. The kidney and prostate gland were

evaluated as systemic target organs in which chronic CO-associated hypoxic stress was reflected by structural remodeling and changes in hypoxia-associated molecular markers.
The integrated analysis therefore compared: baseline age-related morphology; morphological changes induced by chronic CO exposure; quantitative morphometric alterations; Ki-67 expression in the lung; HIF-1α expression in the kidney and prostate; structural changes following phytocorrection.
This design allowed the morphological response of three anatomically and functionally different organs to chronic CO exposure to be evaluated within a common experimental framework.

Results and Discussion

3.1. Chronic CO exposure induces pronounced morphological remodeling in the lung

The control lungs demonstrated preserved alveolar architecture, with relatively uniform alveolar distribution and thin continuous alveolar septa. In the final-age 3-month control group, the mean alveolar area was 2480±95 μm², alveolar diameter was 56.4±2.1 μm, alveolar number was 22.1±0.8 per field, alveolar wall thickness was 4.5±0.2 μm, and relative alveolar volume was 67.8±1.9%.
In the final-age 9-month control group, the corresponding values were 2875±104 μm², 63.1±2.4 μm, 19.2±0.7 alveoli per field, 5.4±0.2 μm, and 72.6±1.7%, respectively. Thus, physiological age-related enlargement of alveolar structures was accompanied by a moderate reduction in alveolar number and an increase in alveolar wall thickness.
Following chronic CO exposure, marked structural remodeling of the alveolar apparatus was observed. In the final-age 3-month CO group, alveolar area increased to 4018±146 μm² and alveolar diameter to 78.6±2.8 μm, while the number of alveoli decreased to 13.9±0.5 per field. Alveolar wall thickness increased to 7.6±0.3 μm, and emphysematous areas accounted for 15.8±0.7%.
In the final-age 9-month CO group, the changes were more pronounced. Mean alveolar area reached 5286±184 μm², alveolar diameter 101.4±3.6 μm, and alveolar wall thickness 10.1±0.4 μm. The number of alveoli decreased to 9.4±0.3 per field, whereas emphysematous areas increased to 26.9±1.1%. These findings demonstrate progressive enlargement and structural destruction of alveolar spaces under chronic CO exposure.

Table 2. Selected morphometric parameters of the lung under chronic CO exposure

Parameter	Control, 3 months	CO, 3 months	Control, 9 months	CO, 9 months
Alveolar area, μm²	2480±95	4018±146	2875±104	5286±184
Alveolar diameter, μm	56.4±2.1	78.6±2.8	63.1±2.4	101.4±3.6
Alveolar number/field	22.1±0.8	13.9±0.5	19.2±0.7	9.4±0.3
Alveolar wall thickness, μm	4.5±0.2	7.6±0.3	5.4±0.2	10.1±0.4
Relative alveolar volume, %	67.8±1.9	81.3±2.1	72.6±1.7	88.7±2.4
Emphysematous area, %	0.8±0.1	15.8±0.7	2.6±0.2	26.9±1.1

3.2. Phytocorrection partially restores lung architecture

Administration of Gulimahsar infusion after chronic CO exposure was associated with substantial improvement of the lung morphometric profile. In the final-age 3-month correction group, emphysematous areas decreased from 15.8±0.7% in the CO group to 7.3±0.3%. Alveolar number increased from 13.9±0.5 to 18.1±0.6 per field, while alveolar wall thickness decreased from 7.6±0.3 to 5.9±0.2 μm.
In the final-age 9-month group, alveolar area decreased from 5286±184 to 4026±152 μm² and emphysematous areas decreased from 26.9±1.1% to 12.9±0.5%. The number of alveoli increased from 9.4±0.3 to 14.6±0.5 per field, while capillary density increased from 7.4±0.3 to 12.1±0.4. These changes indicate partial restoration of the alveolar–capillary structural organization.

Table 3. Effect of Gulimahsar correction on selected lung morphometric parameters

Parameter	CO, 3 months	CO + Gulimahsar, 3 months	CO, 9 months	CO + Gulimahsar, 9 months
Alveolar area, μm²	4018±146	—	5286±184	4026±152

Alveolar number/field	13.9±0.5	18.1±0.6	9.4±0.3	14.6±0.5
Alveolar wall thickness, μm	7.6±0.3	5.9±0.2	10.1±0.4	—
Emphysematous area, %	15.8±0.7	7.3±0.3	26.9±1.1	12.9±0.5
Capillary density	—	—	7.4±0.3	12.1±0.4

Immunohistochemical analysis of Ki-67 provided additional evidence of tissue response to correction. In the 3-month Gulimahsar group, the Ki-67-positive cell fraction was 6.7±0.3%, representing a decrease compared with the chronic CO group while remaining above the control level. In the 9-month correction group, the Ki-67 index was 5.1±0.3%, with a more normalized distribution of immunopositive cells.

3.3. Chronic CO exposure produces hypoxic-dystrophic remodeling in the kidney

Histological examination demonstrated that chronic CO exposure caused structural changes predominantly in the renal cortex. In the 3-month final-age CO group, glomerular capillary loops showed irregular arrangement, partial retraction of the glomerular tuft, enlargement of the Bowman–Shumlyansky capsule space, and an increase in mesangial matrix. Proximal tubular epithelial cells demonstrated hydropic and granular dystrophic changes, cytoplasmic vacuolization, and occasional desquamation.

These changes became more pronounced in the 9-month final-age animals. Glomerular tuft retraction, enlargement of the capsular space, tubular epithelial dystrophy, interstitial edema, and microcirculatory disturbances were more evident, indicating age-associated intensification of chronic CO-related renal remodeling.

3.4. HIF-1α expression is increased in the kidney and prostate following chronic CO exposure

Digital immunohistochemical analysis demonstrated a common hypoxia-associated response in the kidney and prostate.

In the kidney, HIF-1α-positive cells increased from 25.88% in the final-age 3-month control group to 30.32% following chronic CO exposure. In the final-age 9-month animals, the proportion increased from 22.81% in controls to 31.73% in the CO group. In the 9-month CO group, analysis of a 0.188-mm² region of interest identified 602 cells, of which 191 (31.73%) were HIF-1α-positive. The corresponding DAB optical density was 0.191 and the H-score was 110.2.

The prostate demonstrated a more pronounced HIF-1α response. In the final-age 3-month control group, 512 of 1171 analyzed cells were positive, corresponding to 43.73%. Following chronic CO exposure, 823 of 1284 cells were positive (64.11%), with a positive cell density of 643 cells/mm². The immunoreactivity distribution was 13.6% weak, 53.2% moderate, and 33.2% strong.

In the final-age 9-month control group, 598 of 1233 cells were HIF-1α-positive (48.49%). Chronic CO exposure increased the proportion to 1026 of 1460 cells (70.27%), with a positive cell density of 801 cells/mm². Among positive cells, 10.8% demonstrated weak, 50.6% moderate, and 38.6% strong immunoreactivity.

Table 4. HIF-1α expression in kidney and prostate tissues

Organ	Age at assessment	Group	HIF-1α-positive cells, %	Cells analyzed, n	Positive cells, n	Positive cell density, cells/mm²
Kidney	3 months	Control	25.88	568	147	798.91
Kidney	3 months	CO	30.32	—	—	—
Kidney	9 months	Control	22.81	—	—	—
Kidney	9 months	CO	31.73	602	191	—
Prostate	3 months	Control	43.73	1171	512	400
Prostate	3 months	CO	64.11	1284	823	643
Prostate	9 months	Control	48.49	1233	598	—
Prostate	9 months	CO	70.27	1460	1026	801

DAB optical density and H-score values were additionally reported for selected digital-analysis regions and are discussed in the text.

3.5. Chronic CO exposure causes marked structural remodeling of the prostate

Morphological examination of the prostate demonstrated progressive acinar–stromal remodeling under chronic CO exposure. The changes included acinar atrophy, dystrophic alterations of the secretory epithelium, reduced secretory activity, interstitial edema, stromal remodeling, and microcirculatory disturbances.

In the 9-month animals, compared with the corresponding control group, acinar diameter decreased by 21.4%, acinar lumen area by 43.3%, secretory epithelial height by 35.0%, and parenchymal fraction by 20.8%. In contrast, the stromal fraction increased by 50.0%, the stroma-to-parenchyma ratio by 88.1%, and vascular diameter by 39.0% ($P<0.05$). The changes were more pronounced in the older animals, indicating progressive structural remodeling of the prostate under chronic CO exposure.

3.6. Phytocorrection is associated with partial recovery of kidney and prostate morphology

Both *Asparagus officinalis* L. oil and Gulimahsar infusion were associated with attenuation of chronic CO-related structural abnormalities in the kidney and prostate.

In the prostate, correction was associated with increased acinar diameter by 6.8–21.4% and increased secretory epithelial height by 20.3–44.5%. The stromal fraction decreased from 39.8–44.1% to 31.6–34.1%, while the parenchymal fraction recovered to 65.9–68.4%. These changes indicate partial restoration of the acinar–stromal relationship following phytocorrection.

The immunohistochemical findings further demonstrated attenuation of the hypoxia-associated response following correction. In the kidney, the analyzed correction groups showed lower proportions of HIF-1α-positive cells and reduced DAB optical density and H-score compared with the corresponding chronic CO-exposed tissue. However, HIF-1α expression was not completely eliminated, indicating partial rather than complete reversal of the hypoxic response.

3.7. Comparative organ response to chronic CO exposure

The integrated analysis demonstrated a common hypoxia-associated response across all three organs but distinct morphological manifestations according to organ structure and function.

The lung showed the most prominent changes in the alveolar–capillary compartment, including alveolar enlargement, septal thickening, emphysematous remodeling, reduced alveolar number, and altered capillary density. The kidney demonstrated glomerular and tubular dystrophic remodeling accompanied by increased HIF-1α expression. The prostate showed acinar–stromal remodeling and the highest proportion of HIF-1α-positive cells among the evaluated tissues.

The increase in HIF-1α-positive cells was particularly pronounced in the prostate, reaching 64.11% in 3-month and 70.27% in 9-month CO-exposed animals, compared with 30.32% and 31.73% in the corresponding renal CO groups. This pattern indicates that although chronic CO exposure produced a common hypoxia-associated molecular response, the magnitude and structural expression of this response were organ-specific.

Taken together, the morphometric, histological, immunohistochemical, and digital pathology findings support an integrated model in which chronic CO exposure is associated with tissue hypoxia, HIF-1α activation, and progressive organ-specific structural remodeling. Phytocorrection was associated with partial restoration of tissue architecture and attenuation of hypoxia-associated changes.

Discussion

The study demonstrates that chronic carbon monoxide exposure produces substantial morphological remodeling in the lung, kidney, and prostate gland, while the magnitude and structural pattern of the response differ according to the functional characteristics of each organ. Despite these organ-specific differences, the findings indicate a common pathophysiological component associated with chronic hypoxic stress.

A particularly important feature of the experimental design was the assessment of animals at two developmental stages. The animals entered the experiment at 2 and 8 months of age and, following the one-month experimental period, were assessed at 3 and 9 months, respectively. This design allowed the effect of chronic CO exposure to be evaluated against age-matched controls while also demonstrating that the older animals generally developed more pronounced structural alterations.

4.1. Chronic CO exposure and organ-specific morphological remodeling

The pulmonary findings indicate that the lung represents a major structural target of chronic CO exposure. In the study, CO exposure was associated with enlargement of alveolar spaces, increased emphysematous areas, thickening and remodeling of alveolar walls, and reduction of alveolar and capillary density.

The increase in alveolar area from approximately 2480 μm² in the 3-month control group to 4018 μm² after chronic CO exposure, together with the increase in emphysematous areas from less than 1% to 15.8%, indicates substantial remodeling of the gas-exchange compartment. The effect became more pronounced in the older animals, in which the alveolar area reached approximately 5286 μm² and emphysematous changes reached 26.9%.

The simultaneous reduction in alveolar number and capillary density is particularly important. Enlargement of individual alveolar spaces accompanied by loss of alveolar structures and reduction of the microvascular component suggests that the observed changes cannot be interpreted simply as physiological enlargement with age. Rather, they indicate structural remodeling of the alveolar-capillary unit under chronic CO-associated stress.

This pattern is consistent with the concept that prolonged impairment of oxygen delivery can disturb the structural equilibrium between alveolar epithelium, interalveolar septa, and pulmonary microcirculation. The findings therefore support the interpretation of chronic CO exposure as a factor capable of producing progressive structural remodeling of pulmonary tissue.

4.2. Renal response to chronic CO-associated hypoxia

The kidney demonstrated a different pattern of injury, predominantly involving the glomerular and tubular compartments.

The morphological findings included retraction of glomerular capillary tufts, widening of the Bowman–Shumlyansky capsule space, reduction of glomerular dimensions, tubular epithelial dystrophic changes, epithelial thinning, luminal enlargement, interstitial edema, and an increase in the interstitial component.

These changes are consistent with the high sensitivity of renal tissue to disturbances in oxygen availability. The renal cortex contains metabolically active tubular epithelial cells that require continuous oxygen delivery, while the renal microcirculatory architecture creates physiologically low-oxygen regions within the parenchyma. Consequently, prolonged disturbances in oxygen transport may result in structural adaptation followed by dystrophic and interstitial remodeling.

The findings are particularly relevant since chronic CO exposure does not simply cause a generalized reduction in oxygen transport; it can result in tissue-level hypoxic stress that is reflected morphologically in organs with high metabolic or microcirculatory requirements. The observed tubular epithelial changes and expansion of interstitial tissue therefore provide structural evidence of chronic hypoxic remodeling.

4.3. HIF-1α as a common indicator of the hypoxic response

One of the most important findings of the study was the increase in HIF-1α expression in both kidney and prostate tissue following chronic CO exposure.

In the kidney, HIF-1α-positive cells increased from approximately 25.88% to 30.32% in the 3-month animals and from 22.81% to 31.73% in the 9-month animals. In the prostate, the corresponding increase was considerably more pronounced, from 43.73% to 64.11% in the 3-month animals and from 48.49% to 70.27% in the 9-month animals.

The parallel increase in HIF-1α expression and structural abnormalities supports the interpretation that chronic hypoxia is an important component of the tissue response to CO exposure.

HIF-1α is a central regulator of cellular adaptation to reduced oxygen availability. Under hypoxic conditions, stabilization of HIF-1α permits activation of transcriptional programs involved in metabolic adaptation, cell survival, angiogenesis, and maintenance of cellular homeostasis. The immunohistochemical findings therefore provide a molecular correlate for the morphological alterations observed in the examined tissues.

The renal findings are consistent with the concept described in the literature that HIF signaling represents an important adaptive mechanism in hypoxic renal tissue. However, persistent activation of hypoxia-responsive pathways may accompany progressive structural remodeling. In the study, the increased HIF-1α immunoreactivity in older animals occurred together with more pronounced morphological abnormalities, suggesting that prolonged hypoxic signaling may accompany progression from adaptive responses toward structural injury.

4.4. Greater HIF-1α response in the prostate

An important comparative finding was the substantially greater increase in HIF-1α-positive cells in the prostate than in the kidney.

The prostate demonstrated an increase from 43.73% to 64.11% in the 3-month group and from 48.49% to 70.27% in the 9-month group. In the latter group, 1026 of 1460 analyzed cells were HIF-1α-positive, and 38.6% of positive cells demonstrated strong staining intensity.

The predominance of HIF-1α immunoreactivity in the secretory epithelial component is biologically relevant as the prostate is characterized by active secretory epithelial metabolism. The increased immunoreactivity observed in the study may therefore reflect activation of hypoxia-responsive mechanisms within metabolically active epithelial cells.

The accompanying morphological changes—acinar remodeling, epithelial dystrophy and atrophy, stromal expansion, interstitial edema, and microcirculatory disturbances—suggest that the increase in HIF-1α was not an isolated molecular event. Instead, it occurred in parallel with structural remodeling of the gland.

4.5. Relationship between morphological injury and hypoxia-associated signaling

The combined findings from the three organs suggest a common sequence of events:

chronic CO exposure → impaired oxygen availability → tissue hypoxic stress → activation of hypoxia-responsive pathways → cellular and structural remodeling.

The exact morphological manifestation of this sequence differed between organs.

In the lung, the response was dominated by alveolar and microvascular remodeling. In the kidney, glomerular, tubular, and interstitial alterations predominated. In the prostate, remodeling of the acinar-secretory apparatus and stromal component was particularly prominent.

Thus, the same systemic exposure produced different morphological phenotypes according to the tissue-specific architecture and functional requirements of each organ.

The comparative results also suggest that HIF-1α can serve as a useful molecular bridge between the exposure condition and morphological remodeling. This is particularly evident in the kidney and prostate, where increased HIF-1α expression accompanied quantitative morphometric abnormalities.

4.6. Age-dependent intensification of chronic CO effects

Another important observation was the greater severity of several abnormalities in the 9-month final-age animals compared with the 3-month final-age animals.

In the lung, the older CO-exposed group demonstrated a larger increase in alveolar area and a substantially greater proportion of emphysematous changes. In the kidney, older animals showed more pronounced glomerular and tubular remodeling. In the prostate, the proportion of HIF-1α-positive cells reached its highest value in the 9-month CO-exposed group.

These findings suggest that age-related differences in tissue organization, metabolic demand, microcirculation, and adaptive capacity may influence the response to chronic CO exposure.

Importantly, the 2/8-month and 3/9-month age designations represent different stages of the same experimental protocol rather than contradictory age groups. The animals were initially 2 and 8 months old and were assessed at 3 and 9 months after one month of exposure. Therefore, the age-dependent comparison reflects the response of younger and older animals to the same duration of chronic CO exposure.

4.7. Effects of phytocorrection

An important finding of this study was that both phytocorrection protocols were associated with measurable morphological improvement.

In the lung, Gulimahsar correction reduced the emphysema area from 15.8% to 7.3% in the younger animals and from 26.9% to 12.9% in the older animals. At the same time, alveolar number increased and, in the older animals, capillary density increased from 7.4 to 12.1 cells/field. These changes indicate partial restoration of the alveolar-capillary structural relationship.

The renal findings demonstrated a similar pattern. Following phytocorrection, glomerular diameter and area increased, tubular epithelial height improved, while the Bowman–Shumlyansky capsule space and interstitial tissue proportion decreased. These changes are consistent with partial restoration of glomerular and tubular architecture.

In the prostate, phytocorrection was associated with increased acinar diameter and secretory epithelial height and a reduction in the relative stromal component. These findings suggest improvement in the parenchymal-stromal relationship and preservation of the secretory apparatus.

Taken together, the morphological results indicate that the corrective preparations did not simply alter a single quantitative parameter but were associated with coordinated changes in several structural components of the target organs.

4.8. Relationship between phytocorrection and HIF-1α expression

The morphological improvement following phytocorrection was accompanied by a reduction in HIF-1α immunoreactivity.

Digital analysis of corrected renal tissue demonstrated lower proportions of HIF-1α-positive cells and lower DAB optical density and H-score values than in the corresponding untreated CO-exposed groups. In one analyzed correction field, 152 of 587 cells were HIF-1α-positive, corresponding to 25.89%, with a DAB optical density of 0.162 and an H-score of 85.6. Another correction dataset demonstrated 21.80% positive cells, a DAB optical density of 0.141, and an H-score of 74.0.

The reduction of HIF-1α expression after correction is important as it occurred together with improvement in tissue architecture. This parallel change supports the interpretation that reduction of hypoxic stress may be associated with structural recovery.

At the same time, HIF-1α expression was not completely eliminated after correction. This is biologically relevant as physiological HIF signaling participates in normal cellular adaptation to fluctuations in oxygen availability. Therefore, the observed pattern is better characterized as normalization or attenuation of excessive hypoxia-associated signaling rather than complete suppression of HIF-1α.

4.9. Comparative effects of Asparagus officinalis oil and Gulimahsar

The two phytocorrection approaches demonstrated broadly similar directions of effect, although some parameters differed between preparations.

The observed differences should, however, be interpreted cautiously as the study was designed primarily to evaluate morphological correction rather than to establish a definitive superiority hierarchy between the two preparations.

The common feature of both interventions was their association with reduced structural damage and attenuation of hypoxia-associated molecular responses. This concordance strengthens the interpretation that the observed improvement was related to correction of the pathological state rather than to an isolated change in a single morphometric parameter.

4.10. Significance of digital pathology in the assessment of chronic CO injury

The use of digital image analysis provided an additional quantitative dimension to the morphological assessment.

Traditional microscopic examination allows identification of tissue injury, but digital analysis permits systematic quantification of tissue areas, cell populations, immunopositive fractions, staining intensity, and cellular density.

In the study, QuPath-based analysis enabled quantitative assessment of HIF-1α-positive cells and pulmonary Ki-67 expression and supported conventional morphometric observations. The agreement between morphological observations and digital measurements strengthens the reproducibility of the findings.

This approach is particularly useful when comparing multiple organs and age groups as the same quantitative principles can be applied across different tissue types. The results therefore demonstrate the practical value of digital pathology for experimental assessment of chronic toxic and hypoxic injury.

4.11. Integrated interpretation and implications

The combined findings support a model in which chronic CO exposure produces a systemic hypoxic challenge that is expressed differently in individual organs.

The lung demonstrates direct structural consequences within the gas-exchange apparatus, whereas the kidney and prostate demonstrate secondary tissue responses characterized by cellular adaptation, microcirculatory disturbances, and parenchymal remodeling.

The increase in HIF-1α in the kidney and prostate provides a molecular indicator of hypoxia-associated adaptation, while the pulmonary Ki-67 response reflects altered cellular proliferative activity during tissue remodeling and repair.

The fact that morphological recovery following phytocorrection was accompanied by attenuation of HIF-1α expression provides additional evidence that the structural abnormalities were linked to hypoxic stress.

Therefore, the findings support the use of an integrated morphological and immunohistochemical approach for characterization of chronic CO-associated tissue injury. Combining conventional histology, quantitative morphometry, immunohistochemistry, and digital pathology allows the structural and molecular dimensions of chronic hypoxic injury to be evaluated simultaneously.

The study demonstrates that chronic CO exposure is associated with organ-specific morphological remodeling in the lung, kidney, and prostate, with more pronounced alterations in the older experimental animals. The associated increase in hypoxia-responsive signaling and the partial normalization of morphological and immunohistochemical parameters following phytocorrection indicate that these changes are at least partly modifiable.

Conclusion

Chronic carbon monoxide exposure induced pronounced and organ-specific morphological remodeling in the lung, kidney, and prostate. The observed changes reflected the systemic consequences of chronic CO-associated oxygen deficiency and were characterized by structural alterations in the respiratory, renal, and glandular tissues.

In the lung, chronic CO exposure was associated with enlargement of alveolar structures, increased alveolar wall thickness, reduction in alveolar number and capillary density, and an increase in emphysematous changes. These alterations indicate impairment of the structural organization of the alveolar–capillary complex and deterioration of the morphological basis of gas exchange.

In the kidney, chronic CO exposure produced glomerular, tubular, and interstitial remodeling, accompanied by changes in morphometric parameters and an increase in hypoxia-associated HIF-1α expression. The findings indicate that the kidney is sensitive to chronic CO-induced oxygen imbalance, with structural changes involving both the glomerular and tubular compartments.

In the prostate, chronic CO exposure was accompanied by remodeling of the acinar–stromal architecture, changes in secretory epithelial characteristics, and increased HIF-1α expression. The proportion of HIF-1α-positive cells increased from 43.73% to 64.11% in the 3-month final-age group and from 48.49% to 70.27% in the 9-month final-age group following chronic CO exposure. These findings support the involvement of tissue hypoxia-associated mechanisms in prostate remodeling under chronic CO exposure.

A common feature across the examined organs was activation of the HIF-1α-associated hypoxic response. The increase in HIF-1α expression, together with organ-specific morphometric alterations, suggests that chronic CO exposure produces a systemic hypoxic burden while the morphological manifestation of this burden depends on the structural and functional characteristics of individual organs.

Age also influenced the severity of the morphological response. Animals assessed at 9 months after the experimental period generally demonstrated more pronounced structural alterations than those assessed at 3 months, particularly in parameters reflecting alveolar remodeling, microvascular changes, and hypoxia-associated responses.

Administration of Qushqonmas (Asparagus officinalis L.) oil and Gulimahsar infusion was associated with partial structural recovery in the examined organs. In the lung, phytocorrection reduced emphysematous changes, improved alveolar organization, and increased capillary density. In the kidney and prostate, correction was associated with improvement of tissue architecture and reduction of hypoxia-associated HIF-1α immunoreactivity. However, HIF-1α expression was not completely eliminated, indicating that phytocorrection produced partial rather than absolute reversal of chronic CO-associated tissue changes.

Overall, the integrated morphological and immunohistochemical findings demonstrate that chronic CO exposure affects the lung, kidney, and prostate through a common hypoxia-associated pathway while producing distinct organ-specific structural patterns. The combined assessment of morphometry, histology, immunohistochemistry, and digital image analysis provides a comprehensive approach for characterizing chronic CO-induced tissue injury and evaluating the morphological effectiveness of phytocorrection.

Author Contributions
All Authors contributed equally.

Conflict of Interest
The authors declared that no conflict of interest

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