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Morphometric and Histological Alterations in the Spleen Induced by Chronic Carbon Monoxide Exposure and the Corrective Effects of Gulimaxsar

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Abstract

Chronic exposure to carbon monoxide (CO) induces hypoxia and is associated with structural and functional alterations in the spleen. This study aimed to evaluate morphometric and histological changes splenic tissue under prolonged CO exposure and to assess the corrective effects of the medicinal plant Gulimaxsar.

Results showed a significant decrease in white pulp area (−33.3%), germinal center diameter (−28.3%), and marginal zone thickness (−34.0%) ($p < 0.05$), along with an increase in red pulp sinusoid diameter (+47.9%), indicating vascular congestion and immunosuppression.

Administration of Gulimaxsar extract resulted in partial restoration of splenic structure, including an increase in white pulp area (+33.8%) and germinal center diameter (+27.6%) ($p < 0.05$), and a reduction in sinusoidal dilation.

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These findings indicate that Gulimaxsar exerts protective effects against CO-induced splenic damage, likely due to its antioxidant and antihypoxic properties.

Keywords:
Oxidative stress, apoptosis, spleen, morphological changes, experimental study.

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Introduction

Recent studies have increasingly emphasized the vulnerability of immune organs to chronic hypoxic and oxidative stress conditions. The spleen, as a central secondary lymphoid organ, plays a crucial role in immune surveillance and systemic inflammatory regulation. Environmental and toxic stressors have been shown to significantly alter splenic architecture and immune cell balance (Ji et al., 2025).

Chronic hypoxia induces oxidative imbalance through excessive generation of reactive oxygen species (ROS), leading to mitochondrial dysfunction and activation of apoptotic pathways (Lira-Mejía et al., 2025). Persistent redox disruption promotes structural remodeling in lymphoid tissues, including depletion of white pulp and sinusoidal dilation in red pulp regions (Zhang et al., 2025).

Oxidative stress is considered a principal mediator of splenic cellular damage. Excess ROS activates pro-apoptotic signaling cascades and disrupts cellular proliferation–apoptosis balance (Manful et al., 2025). Recent mechanistic analyses further confirm that redox imbalance contributes to inflammatory activation and tissue degeneration across organ systems (Antioxidants, 2025).

Experimental toxicology models demonstrate that prolonged exposure to environmental toxins results in lymphoid depletion, stromal disorganization, and increased apoptotic indices in splenic tissue (Ning et al., 2024). Similar findings were reported in oxidative injury models where structural damage of the spleen was associated with significant morphometric decline in germinal centers and marginal zones (Khatun et al., 2024).

Morphometric approaches have become increasingly important in assessing immune organ status. Quantitative evaluation of splenic compartments provides objective evidence of structural impairment under stress conditions (Zhang et al., 2025). Alterations in white pulp area and sinusoidal expansion are regarded as indicators of suppressed immune reactivity.

Parallel to these findings, growing attention has been directed toward phytotherapeutic correction strategies. Plant-derived antioxidants have demonstrated strong cytoprotective properties by reducing lipid peroxidation and stabilizing mitochondrial membranes (Muscolo et al., 2024). Bioactive phytochemicals are capable of modulating inflammatory mediators and restoring antioxidant enzyme activity (Venkataraman et al., 2025).

Supplementation studies further support the therapeutic role of antioxidants in immune organ protection. Melatonin administration significantly attenuated oxidative damage and apoptotic activation in spleen and thymus tissues (Ning et al., 2024). Similarly, vitamin E was shown to reduce histological damage and improve splenic structural integrity under oxidative stress conditions (Khatun et al., 2024).

Taken together, contemporary high-impact studies indicate that chronic hypoxic and toxic exposure induces significant oxidative and morphostructural alterations in splenic tissue. At the same time, antioxidant-based phytotherapeutic interventions demonstrate promising restorative potential. Nevertheless, despite expanding research on oxidative stress mechanisms, limited data exist regarding chronic carbon monoxide–induced morphometric changes in the spleen and their correction using regional medicinal plants. Therefore, further investigation in this field remains scientifically justified and relevant.

Despite extensive research on hypoxia-induced oxidative stress and its impact on immune organs, limited data are available regarding the morphometric alterations of the spleen under chronic carbon monoxide exposure. Furthermore, the potential corrective effects of regional medicinal plants, particularly Gulimaxsar, remain insufficiently explored.

Gulimaxsar is known to possess antioxidant and immunomodulatory properties; however, its role in preventing or reversing carbon monoxide–induced splenic damage has not been adequately investigated. Therefore, this study aims to fill this gap by evaluating both the structural changes in the spleen under chronic CO exposure and the therapeutic potential of Gulimaxsar.

Related Work

The experimental study was conducted on 250 outbred white laboratory rats of both sexes aged 1 and 7 months. All animals were kept under standard vivarium conditions with free access to food and water. Prior to the experiment, the animals were quarantined for one week and clinically examined to exclude any somatic or infectious diseases.
The study was carried out in two sequential stages:
(1) induction of chronic CO intoxication;
(2) corrective treatment of intoxication-induced changes.
At the first stage, animals were randomly divided into two groups (n = 250):
Group I (control, n = 60): intact animals;
Group II (CO exposure, n = 190): animals subjected to chronic carbon monoxide intoxication.
Chronic CO exposure was performed in a sealed exposure chamber. Carbon monoxide was generated by incomplete combustion of carbon-containing fuel under controlled oxygen-limited conditions. The generated gas mixture was delivered into the exposure chamber through a tubing system.
CO concentration in the chamber was maintained within the range of 0.01–0.05 mg/L and continuously monitored using a portable gas analyzer at 10–15 minute intervals. Minimal ventilation was provided to prevent critical oxygen depletion. Animals were exposed to CO for 1 month and 14 days, modeling chronic low-dose intoxication.
During the experiment, 10 animals died due to CO exposure (4 in the 1-month group and 6 in the 7-month group).
Corrective treatment. At the second stage, surviving animals (n = 120) were divided into two groups:
Group III (CO + asparagus oil, n = 60): animals received intragastric administration of asparagus oil (0.1 ml/day, 1:9 solution) for 14 days;
Group IV (CO + Gulimaxsar, n = 60): animals received intragastric administration of Gulimaxsar extract (0.1 ml/day, aqueous extract 1:10) for 14 days.
The Gulimaxsar extract was prepared by mixing dried plant material with distilled water (1:10), heating at 80–90°C in a water bath for 30 minutes, followed by filtration and cooling to room temperature before administration.
Monitoring and analysis. Throughout the experiment, animal behavior and physiological condition were monitored. At the end of the experiment, spleen tissues were collected for morphometric and histological analysis using standard staining methods.
Statistical analysis was performed using SPSS software (version XX, IBM Corp., USA). Data were expressed as mean ± standard error of the mean (SEM). Comparisons between groups were conducted using Student's t-test and one-way analysis of variance (ANOVA) where appropriate. A p-value of less than 0.05 was considered statistically significant.

Results, Performance Evaluation, and Discussion

Histological examination of splenic tissue in the control group revealed preserved structural organization, with clearly distinguishable white and red pulp, well-defined periarterial lymphoid sheaths (PALS), prominent germinal centers, and intact marginal zones. The red pulp showed normal sinusoidal architecture without vascular congestion. In the Gulimaxsar-treated group, partial restoration of splenic architecture was observed, characterized by increased white pulp volume, improved definition of germinal centers, enhanced lymphocyte density, recovery of marginal zone thickness, and reduced red pulp congestion. As a result of chronic exposure to CO, the body weight of 6-month-old laboratory animals ranged from 160 to 230 g, with a mean of 201.8 ± 7.36 g. The absolute organ weight ranged from 0.44 to 0.76 g, with a mean of 0.59 ± 0.03 g. The weight index ranged from 0.217% to 0.322%, with a mean of 0.295 ± 0.01%.
Spleen length ranged from 25.4 to 33.8 mm, with a mean of 29.3 ± 0.77 mm. Spleen width ranged from 4.0 to 6.4 mm, with a mean of 5.16 ± 0.22 mm. Spleen thickness ranged from 2.0 to 3.8 mm, with a mean of 3.02 ± 0.16 mm (Figure 1).

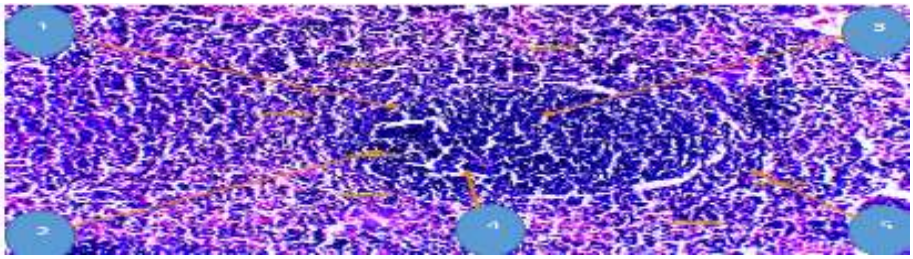

Figure 1. Morphometry of the spleen of a 6-month-old white outbred rat after chronic CO exposure. Staining: Hematoxylin-eosin. Magnification: 20×10. 1 – lymphatic nodule, 2 – periarterial region, 3 – germinal center, 4 – mantle zone, 5 – marginal zone.

According to our data, in 6-month-old white rats, chronic exposure to CO resulted in the relative area of the spleen's white pulp ranging from 12.2% to 21.4%, with a mean of 15.97 ± 0.66%. The growth rate compared to control 6-month-old rats was 15.23%. The relative area of connective tissue elements ranged from 4.8% to 6.0%, with a mean of 6.43 ± 0.11% (relative to the total area of the spleen section). The diameter of the PALS (periarterial lymphatic sheath) ranged from 115.6 mkm to 123.8 mkm, with a mean of 120.83 ± 0.75 mkm. The growth rate compared to control 6-month-old rats was 22.64%. The diameter of the lym-phatic nodules ranged from 365.3 mkm to 430.7 mkm, with a mean of 394.87 ± 6.1 mkm. The growth rate compared to control 6-month-old rats was 101.2%. Germinal centers were identified within the lymphatic nodules. The diameter of germinal centers ranged from 92.3 mkm to 120.8 mkm, with a mean of 103.09 ± 2.44 mkm. The percentage ratio of primary to secondary lymphatic nodules was 44% and 52%, respec-tively. Lymphatic nodules were predominantly circular, oval, or elongated (90.8%) and irregular in shape (8.0%).

The width of the mantle zone in the spleen lym-phatic nodules ranged from 37.4 mkm to 46.6 mkm, with a mean of 42.64 ± 0.84 mkm. The width of the marginal zone ranged from 70.2 mkm to 75.8 mkm, with a mean of 73.81 ± 0.91 mkm. The width of the periarterial region ranged from 77.2 mkm to 85.4 mkm, with a mean of 82.32 ± 0.84 mkm.

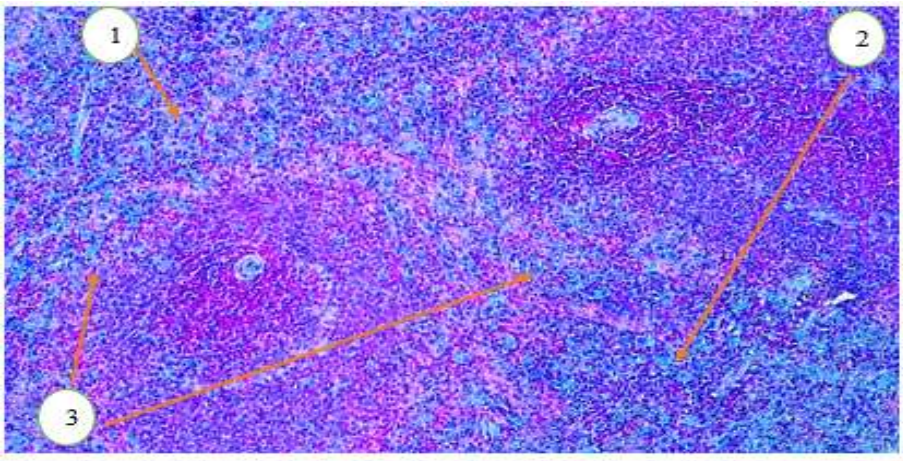

Figure 2. Microscopic appearance of the spleen of a 6-month-old white outbred rat after chronic CO exposure. Staining: Alcian blue. Magnification: 20×10. 1 – densely stained capsule zone, 2 – medullary zone, 3 – trabeculae and adipose tissue between lobules.

In the Gulimaxsar-treated group, partial restoration of splenic architecture was observed, characterized by increased white pulp volume, improved definition of germinal centers, and enhanced lymphocyte density, indicating recovery of splenic structure.

Table 1. Morphometric parameters of the spleen (Mean ± SEM)

Parameter	Control	CO Exposure	CO + Gulimaxsar	% Change (CO vs Control)	% Change (Correction vs CO)
White pulp area (%)	32.4 ± 1.8	21.6 ± 1.4*	28.9 ± 1.6#	↓33.3%	↑33.8%
Germinal center diameter (mkm)	185.2 ± 9.4	132.7 ± 7.8*	169.3 ± 8.1#	↓28.3%	↑27.6%

Parameter	Control	CO Exposure	CO + Gulimaxsar	% Change (CO vs Control)	% Change (Correction vs CO)
Marginal zone thickness (mkm)	42.6 ± 2.3	28.1 ± 1.9*	37.4 ± 2.1#	↓34.0%	↑33.1%
Red pulp sinusoid diameter (mkm)	24.8 ± 1.2	36.7 ± 1.5*	29.3 ± 1.4#	↑47.9%	↓20.1%

*p < 0.05 vs control
#p < 0.05 vs CO group

Chronic CO exposure significantly reduced white pulp area by 33.3% and germinal center diameter by 28.3% compared to controls. Marginal zone thickness decreased by 34.0%. Conversely, red pulp sinusoid diameter increased by 47.9%, indicating vascular congestion.
Administration of Gulimaxsar extract resulted in statistically significant restoration of white pulp area (+33.8%) and germinal center diameter (+27.6%) compared to the CO group.
Immunohistochemical analysis revealed decreased CD3 and CD20 expression in the CO-exposed group, indicating suppression of T- and B-lymphocyte populations. Ki-67 proliferation index significantly declined, reflecting reduced lymphoproliferative activity. Conversely, Caspase-3 expression increased, suggesting enhanced apoptotic activity. Bcl-2 expression was reduced in lymphoid follicles, indicating compromised anti-apoptotic regulation.
In the correction group, CD3 and CD20 expression levels were significantly restored. Ki-67 index increased compared to the CO group, indicating reactivation of proliferative processes. Caspase-3 positivity decreased, and Bcl-2 expression improved, suggesting restoration of apoptosis–proliferation balance.

Discussion

Chronic exposure to carbon monoxide induces tissue hypoxia due to carboxyhemoglobin formation, impairing oxygen delivery to peripheral organs. The spleen, being a highly vascularized immunological organ, is particularly sensitive to hypoxic injury.
The observed reduction in white pulp area and germinal center diameter suggests suppression of lymphoproliferative activity. Lymphoid depletion may reflect impaired T- and B-cell maturation under prolonged hypoxic stress. Enlargement of red pulp sinusoids and vascular congestion indicates circulatory disturbances and compensatory vasodilation.
These findings are consistent with previous studies demonstrating the immunosuppressive effects of chronic hypoxia on lymphoid organs (Rahman et al., 2025; Chen et al., 2024).
Correction with Gulimaxsar plant extract demonstrated notable morphostructural improvement. The partial restoration of white pulp and germinal centers indicates reactivation of lymphoid proliferation. The reduction of sinusoidal dilation suggests improvement of microcirculation.
The protective effect of Gulmansar may be attributed to its antioxidant and cytoprotective properties, reducing oxidative stress induced by CO exposure. By limiting hypoxia-mediated cellular damage and apoptosis, the extract supports structural recovery of splenic tissue.
Overall, these findings suggest that chronic CO exposure is associated with significant morphometric and structural alterations in the spleen, while phytotherapeutic correction with Gulimaxsar may contribute to partial restoration of immune architecture.

Conclusion

Chronic exposure to CO is associated with significant morphometric and histological alterations in the spleen, including a reduction in white pulp area, decreased germinal center size, and vascular changes. These alterations reflect impaired immunomorphological function under hypoxic conditions.
Administration of Gulimaxsar extract resulted in partial restoration of splenic structure, indicating its potential protective and corrective effects, likely associated with its antioxidant and antihypoxic properties.

Author Contributions
All Authors contributed equally.

Conflict of Interest
The authors declare that there are no conflicts of interest.

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