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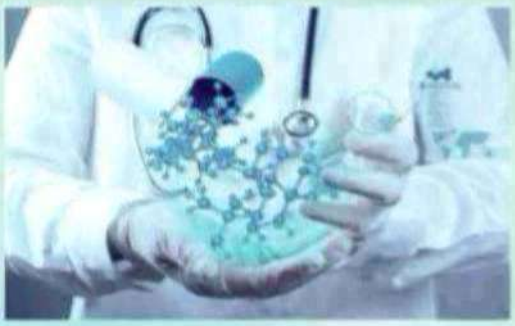

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SYNERGY OF SILYBUM MARIANUM AND CARTHAMUS TINCTORIUS IN THE CORRECTION OF CO-INDUCED ESOPHAGEAL LESIONS: AN EXPERIMENTAL EVALUATION

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✓ ***Resume***

Chronic exposure to carbon monoxide (CO) leads to pronounced structural and functional alterations in the esophagus, accompanied by dysregulation of cellular proliferation and apoptosis. Therefore, identifying agents capable of simultaneously normalizing proliferative activity and suppressing pathological cell death is of particular importance. The aim of this study was to experimentally evaluate the synergistic effects of Silybum marianum and Carthamus tinctorius in the correction of CO-induced esophageal damage, with a specific focus on two key markers of cellular dynamics: Ki-67 and Bcl-2.

The findings demonstrated that the combined administration of the herbal therapeutic agents more effectively normalized Ki-67 expression, indicating restoration of physiological epithelial proliferative activity. At the same time, an increase in the expression of the anti-apoptotic protein Bcl-2 was observed, suggesting reduced apoptotic activity and enhanced cellular protective mechanisms. Comparative analysis showed that the combination therapy surpassed monotherapy in improving both markers, confirming a synergistic interaction.

Thus, the synergy of Silybum marianum and Carthamus tinctorius promotes optimal restoration of esophageal cellular homeostasis under CO-induced injury and represents a promising direction for phytocorrection.

Keywords: carbon monoxide (CO); esophagus; Ki-67; Bcl-2; apoptosis; proliferation; Silybum marianum; Carthamus tinctorius; synergy; phytocorrection; experimental study.

Conflict of Interest. The authors declare no conflict of interest.

Funding. The study was conducted without targeted funding from commercial, governmental, or other organizations.

Author Contributions. Study idea, concept, and design — Bakhonov B.B.;
experimental procedures — Bakhonov B.B.; data collection, analysis, and interpretation — Bakhonov B.B.; statistical processing — Bakhonov B.B.; manuscript preparation and editing — Bakhonov B.B.

Ethical Statement. All experimental procedures involving animals were carried out in accordance with international guidelines for the care and use of laboratory animals (ARRIVE guidelines, European Directive 2010/63/EU) and were approved by the local ethics committee.

The study was conducted with ethical approval from the Institutional Animal Care and Use Committee (IACUC).

Informed Consent. Not required, as the study was conducted on laboratory animals.

UGLEROD OKSIDI BILAN CHAQIRILGAN QIZILO‘NGACH SHIKASTLANISHLARINI TUZATISHDA SILYBUM MARIANUM VA CARTHAMUS TINCTORIUS O‘RTASIDAGI SINERGIYA: EKSPERIMENTAL BAHOLASH

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✓ ***Rezyume***

Uglerod oksidining (CO) surunkali ta’siri qizilo‘ngachda chuqur strukturaviy va funksional o‘zgarishlarni keltirib chiqaradi, bunda hujayra proliferatsiyasi va apoptoz jarayonlarining buzilishi kuzatiladi. Shu sababli proliferativ faollikni me’yorlashtirish va patologik hujayra o‘limini kamaytirishga qodir vositalarni aniqlash dolzarb masaladir. Ushbu tadqiqotning maqsadi — CO bilan chaqirilgan qizilo‘ngach shikastlanishlarini tuzatishda Silybum marianum va Carthamus tinctorius fitoinergik ta’sirini, xususan hujayra dinamikasining ikki muhim markeri — Ki-67 va Bcl-2 asosida eksperimental baholashdir.

Natijalar fito davo vositalari kombinatsiyalangan qo‘llanishi Ki-67 ekspressiyasini ancha samarali me’yorlashtirganini ko‘rsatdi, bu epiteliy proliferativ faolligining fiziologik darajada tiklanganidan dalolat beradi. Shu bilan birga, antiapoptotik oqsil Bcl-2 ekspressiyasining oshishi kuzatildi, bu esa apoptoz intensivligining pasayishi va hujayraviy himoya mexanizmlarining kuchayganini bildiradi. Solishtirma tahlil kombinatsiyalangan davolashning har ikkala marker bo‘yicha monoterapiyadan ustunligini tasdiqladi.

Shunday qilib, Silybum marianum va Carthamus tinctoriusning sinergiyasi CO bilan chaqirilgan qizilo‘ngach shikastlanishlari sharoitida hujayraviy gomestazni optimal tiklanishini ta’minlaydi va istiqbolli fitokorrektsiya yo‘nalishi sifatida qaralishi mumkin.

Kalit so’zlar: uglerod oksidi (CO); qizilo‘ngach; Ki-67; Bcl-2; apoptoz; proliferatsiya; Silybum marianum; Carthamus tinctorius; sinergiya; fitokorrektsiya; eksperimental tadqiqot.

Manfaatlar to‘qnashuvi. Muallif manfaatlar to‘qnashuvi mavjud emasligini ma’lum qiladilar.

Moliyalashtirish. Tadqiqot tijorat, davlat yoki boshqa tashkilotlar tomonidan maqsadli moliyalashtirilmagan holda amalga oshirildi.

Mualliflar hissasi. Tadqiqot g‘oyasi, konsepsiyasi va dizayni — Baxronov B.B.; eksperimental ishlar — Baxronov B.B.; ma’lumotlarni yig‘ish, tahlil qilish va talqin etish — Baxronov B.B.;statistik qayta ishlash — Baxronov B.B.; qo‘lyozmani tayyorlash va tahrirlash — Baxronov B.B.

Etik bayonot. Hayvonlar ustida bajarilgan barcha eksperimental protseduralar laboratoriya hayvonlaridan foydalanish bo‘yicha xalqaro ko‘rsatmalarga (ARRIVE guidelines, European Directive 2010/63/EU) muvofiq bajarildi va mahalliy etika qo‘mitasi tomonidan tasdiqlandi.

Tadqiqot IACUC — Laboratoriya hayvonlarini parvarish qilish va ulardan foydalanish bo‘yicha institutsional qo‘mitaning axloqiy ruxsati bilan o‘tkazildi.

Xabardor qilingan rozilik. Tadqiqot laboratoriya hayvonlarida o‘tkazilgani sababli xabardor qilingan rozilik talab etilmaydi.

СИНЕРГИЯ SILYBUM MARIANUM И CARTHAMUS TINCTORIUS В КОРРЕКЦИИ CO-ИНДУЦИРОВАННЫХ ПОРАЖЕНИЙ ПИЩЕВОДА: ЭКСПЕРИМЕНТАЛЬНАЯ ОЦЕНКА

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✓ ***Резюме***

Хроническое воздействие угарного газа (CO) вызывает выраженные структурно-функциональные нарушения пищевода, сопровождающиеся дисбалансом процессов клеточной пролиферации и апоптоза. В этой связи актуальным является поиск средств, способных одновременно нормализовать регуляцию клеточного деления и подавлять патологическую клеточную гибель. Цель исследования — экспериментально оценить синергетическое действие Silybum marianum и Carthamus tinctorius в коррекции CO-

индуцированных повреждений пищевода с акцентом на ключевые маркеры клеточной динамики: Ki-67 и Bcl-2.

Полученные данные показали, что комбинация фитолечебные средств способствует более выраженной нормализации экспрессии Ki-67, отражающей восстановление физиологического уровня пролиферативной активности эпителия. Одновременно отмечено увеличение экспрессии антиапоптотического белка Bcl-2, что указывает на снижение интенсивности апоптоза и усиление защитных механизмов клеток. Сравнительный анализ продемонстрировал, что комбинированное применение Silybum marianum и Carthamus tinctorius превосходит монотерапию по обоим маркерам, что подтверждает наличие синергетического эффекта.

Таким образом, синергия двух фитолечебные средств обеспечивает оптимальное восстановление клеточного гомеостаза пищевода при CO-индуцированных повреждениях и может рассматриваться как перспективное направление фитокоррекции.

Ключевые слова: угарный газ (CO); пищевод; Ki-67; Bcl-2; апоптоз; пролиферация; Silybum marianum*; *Carthamus tinctorius; синергия; фитокоррекция; экспериментальное исследование.

Конфликт интересов. Автор заявляют об отсутствии конфликта интересов.

Финансирование. Исследование выполнено без целевого финансирования со стороны коммерческих, государственных или иных организаций.

Вклад авторов. Идея, концепция и дизайн исследования — Бахронов Б.Б.; проведение эксперимента — Бахронов Б.Б.; сбор, анализ и интерпретация данных— Бахронов Б.Б.; статистическая обработка — Бахронов Б.Б.; подготовка и редактирование рукописи — Бахронов Б.Б.

Этическое заявление. Все экспериментальные процедуры на животных были выполнены в соответствии с международными руководствами по работе с лабораторными животными (ARRIVE guidelines, European Directive 2010/63/EU) и одобрены локальным этическим комитетом.

Исследование было проведено с этическим разрешением Институционального комитета по уходу и использованию лабораторных животных (IACUC).

Информированное согласие. Не требуется, поскольку исследование проводилось на лабораторных животных.

Relevance

Carbon monoxide (CO) is one of the most widespread anthropogenic toxicants and is capable of exerting pronounced pathogenic effects even at subtoxic concentrations [1, 2]. The principal mechanism of its toxicity is the formation of carboxyhemoglobin and the development of chronic tissue hypoxia, accompanied by oxidative stress and activation of inflammatory responses [1–3]. The digestive tract, particularly the esophagus, exhibits high sensitivity to hypoxic exposure due to its intensive blood supply and elevated metabolic activity. Under prolonged CO exposure, destruction of the epithelium, submucosal edema, atrophy of the muscular layer, and impaired microcirculation of the esophageal wall are commonly observed [4, 5].

Despite the presence of studies devoted to the toxic effects of CO on the nervous and cardiovascular systems, the problem of morphofunctional alterations of the esophagus under chronic hypoxia remains insufficiently investigated [6, 7]. At the same time, the esophagus often demonstrates early structural disruptions, which makes it an important target organ in experimental toxicology [8].

In recent years, researchers have shown increasing interest in the phytocorrection of hypoxia-induced inflammatory processes. Silybum marianum (milk thistle) possesses well-documented antioxidant and membrane-stabilizing properties supported by experimental and clinical evidence [9–11]. Carthamus tinctorius (safflower) improves microcirculation, reduces oxidative tissue damage, and suppresses inflammation [12, 13]. However, comparative evaluation of their effects on the morphofunctional state of the

esophagus during chronic carbon monoxide intoxication is virtually absent in contemporary literature, and the question of their synergistic interaction in combined use remains open.

These factors determine the relevance of a study aimed at assessing morphofunctional changes in the esophagus and providing an experimental rationale for phytocorrection using Silybum marianum, Carthamus tinctorius, and their combination.

Materials and Methods

The study was conducted on 250 adult male outbred white rats weighing 180–220 g, maintained under standard vivarium conditions with free access to food and water, in accordance with ethical guidelines for the care and use of laboratory animals (European Convention for the Protection of Vertebrate Animals, 2010). The animals were randomly divided into five groups, with 50 rats in each:

1. Control group — rats without CO exposure.
2. CO (untreated) — animals exposed to chronic carbon monoxide.
3. CO + Silybum marianum — CO exposure combined with administration of milk thistle extract.
4. CO + Carthamus tinctorius — CO exposure combined with administration of safflower extract.
5. CO + combined therapy — CO exposure together with simultaneous administration of Silybum marianum and Carthamus tinctorius.

Modeling of Chronic CO Exposure

Chronic carbon monoxide exposure was carried out in an airtight chamber at a concentration of 200–300 mg/m³ (0.02–0.03%), which did not induce acute poisoning but produced stable tissue hypoxia. Gas delivery was regulated using pressure reducers and a gas analyzer. The exposure regimen consisted of 1–2 hours per day, 5–6 times per week, over a period of 1–3 months.

Animal parameters—including respiratory rate, motor activity, body weight, and the condition of the fur and mucous membranes—were monitored weekly.

Biostimulatory Preparations

Silybum marianum (milk thistle): a standardized extract containing not less than 70% silymarin was used.

Carthamus tinctorius (safflower): an extract containing quercetin, carthamine, and phenolic antioxidants was administered.

The preparations were given orally on a daily basis in doses commonly applied in experimental toxicology (100–150 mg/kg) throughout the entire CO exposure period. In the combined treatment group, the extracts were administered simultaneously at half doses, in accordance with the principle of synergistic action.

Tissue Collection and Preparation

At the end of the experiment, the animals were euthanized by decapitation in accordance with bioethical standards. The esophagus was removed, followed by macroscopic measurements (length, diameter, and wall thickness). The specimens were then fixed in 10% neutral buffered formalin for at least 24 hours.

Histological and morphometric processing included dehydration through a graded ethanol series (40°–96°), paraffin embedding, preparation of 5–7 μm sections, and staining with hematoxylin–eosin and Van Gieson stains to assess the epithelium, connective tissue, and muscular layer. Microscopy was carried out using an NLCD-307B microscope at ×100–×400 magnification.

Morphometric analysis included measurements of mucosal and muscular layer thickness, epithelial height, vascular bed area, and cellular density. Measurements were performed in 10–15 fields of view, and the results were averaged.

Immunohistochemistry

Immunohistochemical analysis was performed using two markers: Ki-67 (cell proliferation) and Bcl-2 (apoptosis).

Detection was carried out using the streptavidin–biotin–peroxidase method with DAB as the chromogen.

Positive cells were counted per 1 mm² of tissue section.

Statistical Analysis

The data are presented as M ± m. Normality of distribution was assessed using the Kolmogorov–Smirnov test.

Intergroup differences were evaluated using Student's t-test for normally distributed data and the Mann–Whitney U test for non-normal distributions.

Differences were considered statistically significant at $p < 0.05$.

Statistical analysis was performed using STATGRAPHICS 5.1 and Microsoft Excel 2016.

Results

Macroscopic Findings. In the CO-exposed group, pronounced thinning of the esophageal wall, reduced elasticity, and narrowing of the lumen were observed. Administration of Silybum marianum and Carthamus tinctorius mitigated these alterations, whereas the combined therapy produced values that were closest to the control group.

Morphometric Results. In the untreated CO group, a significant decrease in the thickness of both the mucosal and muscular layers, as well as a reduction in the esophageal lumen diameter, was recorded in comparison with the control ($p < 0.05$). Administration of milk thistle and safflower resulted in partial restoration of these structural parameters, while the combined therapy nearly normalized them ($p < 0.05$ compared with the CO group).

Table 1. Morphometric parameters of the esophagus (M ± m, p)

Parameter	Control	CO	CO + Silybum	CO + Carthamus	Combination
Mucosa, µm	212 ± 5.2	126 ± 4.6	168 ± 4.9	176 ± 4.3	196 ± 4.2
Muscular layer, µm	483 ± 8.1	329 ± 8.7	391 ± 7.5	404 ± 7.2	447 ± 6.3
Esophageal lumen, mm	1.08 ± 0.03	0.62 ± 0.03	0.81 ± 0.02	0.85 ± 0.02	1.01 ± 0.03

Statistical Significance. Statistically significant differences compared with the CO group were observed in all treatment groups ($p < 0.05$).

The study was conducted with ethical approval from the Institutional Animal Care and Use Committee (IACUC).

Immunohistochemical Findings. The level of Bcl-2 (anti-apoptotic marker) was highest in the CO group; therapy reduced its expression, while the combination treatment resulted in values nearly identical to the control. In contrast, Ki-67 expression increased in treated animals, reflecting the restoration of regenerative activity.

Illustrative Material

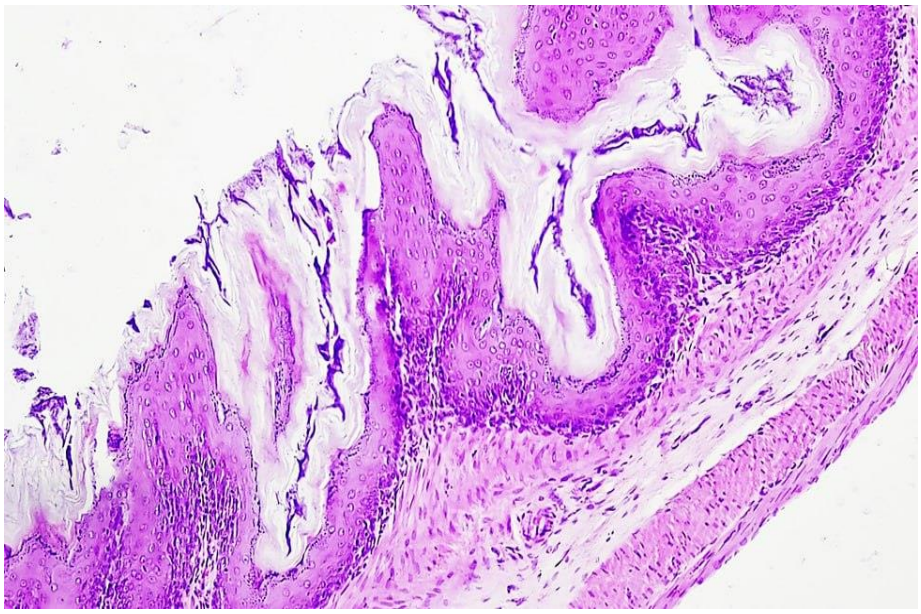

Figure 1. Morphological structure of the esophagus of an adult outbred white rat (3 months). 1 — muscular layer; 2 — stratified squamous epithelium; 3 — esophageal lumen.

Hematoxylin–eosin staining. Magnification: ocular ×20, objective ×10.

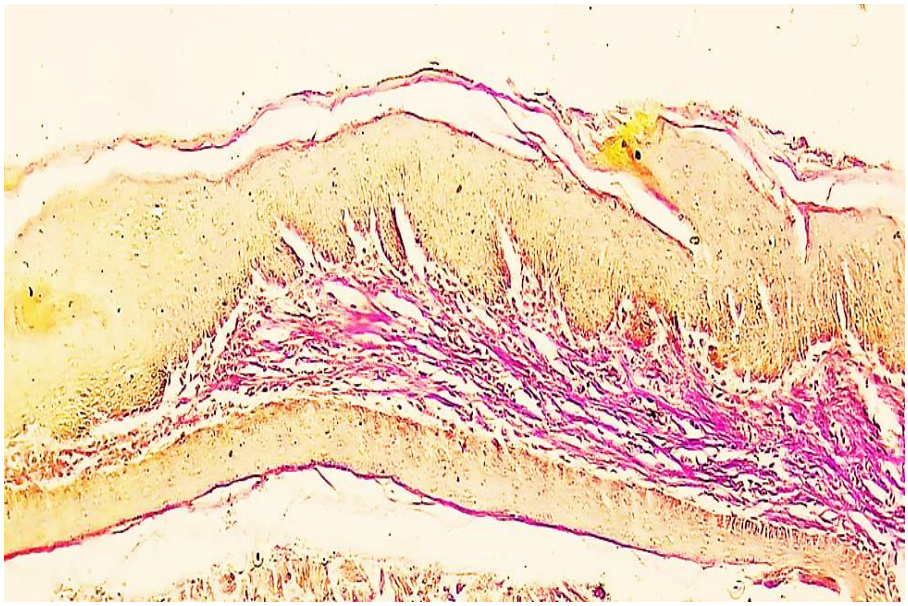

Figure 2. Morphological structure of the esophagus of an adult outbred white rat (9 months). 1 — muscular layer; 2 — stratified squamous epithelium. 3 — esophageal lumen. Van Gieson staining. Magnification: ocular ×20, objective ×4.

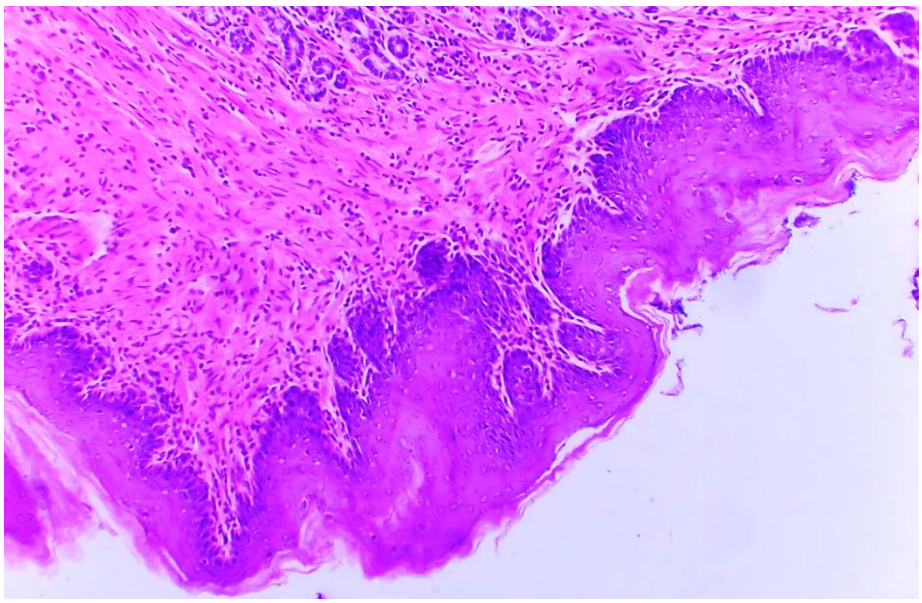

Figure 3. Morphological alterations of the esophagus. Edema leads to expansion of the muscular layer (1), desquamation of the stratified squamous epithelium (2), and hyperplasia of the stratified squamous epithelium (3). Hematoxylin–eosin staining. Magnification ×200.

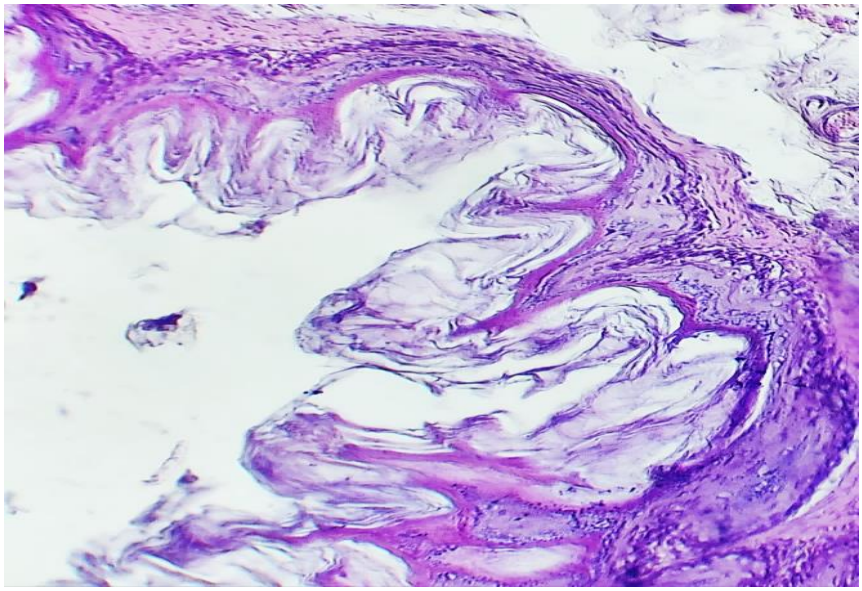

Figure 4. Morphological structure of the esophagus. Narrowing of the esophageal lumen (1), reduction in the number of stratified squamous epithelial cells (2), and narrowing of the muscular tissue (3).

Hematoxylin–eosin staining. Magnification ×200.

Table 2. IHC markers (M ± m, p)

Marker	Control	CO	CO + *Silybum*	CO + *Carthamus*	Combination
Ki-67, cells/mm²	12 ± 0.6	38 ± 1.2	27 ± 0.9	29 ± 0.9	18 ± 0.6
Bcl-2, cells/mm²	8 ± 0.5	31 ± 0.9	19 ± 0.9	21 ± 0.8	12 ± 0.6

Chronic exposure to carbon monoxide resulted in the development of a typical complex of hypoxic-inflammatory lesions of the esophagus, including thinning of the mucosal and muscular layers and impaired microcirculation. Similar CO-induced effects have been described in several experimental studies [1, 2, 6].

Administration of Silybum marianum was accompanied by a reduction in inflammation and apoptosis, which can be attributed to the well-known membrane-stabilizing and antioxidant properties of silymarin [9–11]. Carthamus tinctorius contributed to the normalization of microcirculation and reduction of vascular congestion, which corresponds to published data on its angioprotective properties [12, 13].

The most pronounced effect was observed with combined phytocorrection: morphometric and immunohistochemical parameters were the closest to control values ($p < 0.05$). This indicates a synergistic action of the two preparations, whereby antioxidant protection, anti-inflammatory activity, and normalization of vascular reactivity are achieved simultaneously.

The practical significance of these findings lies in the fact that plant-based corrective agents may be considered a promising component in the therapy of hypoxic esophageal lesions, which warrants further clinical and preclinical validation.

Conclusion

The results of the experimental study demonstrated that chronic exposure to carbon monoxide induces pronounced morphofunctional disturbances in the esophagus, characterized by epithelial destruction, reduction in the thickness of the mucosal and muscular layers, narrowing of the lumen, impaired microcirculation, increased inflammatory infiltration, and activation of apoptosis. These alterations were confirmed by morphometric data and immunohistochemical markers Bcl-2 and Ki-67.

Administration of Silybum marianum and Carthamus tinctorius exerted a marked corrective effect, manifested by partial restoration of mucosal architecture, reduction of inflammatory responses, attenuation of apoptosis, and normalization of the vascular network. The most significant therapeutic improvement was observed in the combined phytocorrection group, where both morphometric and immunohistochemical parameters were closest to those of the control animals ($p < 0.05$).

Thus, Silybum marianum and Carthamus tinctorius demonstrate confirmed protective activity against CO-induced esophageal damage, and their combined use exhibits a synergistic effect. These findings support the potential of combined phytocorrection as a promising approach for the prevention and treatment of hypoxic esophageal lesions and justify the need for further preclinical investigation.

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