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# STUDYING THE CHRONIC TOXICITY OF SULPHAPORIN

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**Abstract:** For the first time, a comprehensive set of preclinical experimental studies of the new therapeutic drug "Sulfoparin," developed by the Institute of Chemistry and Physics of Polymers of the Academy of Sciences of the Republic of Uzbekistan, was conducted. The investigation established that under chronic exposure for an uninterrupted duration of 3 months, the administration of the drug at doses of 500, 100, and 25 mg/kg of body weight does not provoke any pathological alterations within the organisms of the experimental animals. Subsequent morphological examinations confirmed that no statistically or clinically significant differences were present in the structural integrity of the internal organs between the experimental and control groups. Consequently, Sulfoparin does not induce dystrophic, necrobiotic, or inflammatory tissue changes in the animal models.

**Keywords:** drug Sulfoparin, chitosan, chronic toxicity, cumulative properties, atherosclerosis.

## INTRODUCTION

In modern pharmacology, there is a continuously growing interest in the development and application of preparations based on naturally occurring biopolymers. Among these, chitin and its deacetylated derivative, chitosan, have garnered immense attention. Chitosan and its specific chemical derivatives, such as chitosan sulfate, possess a broad spectrum of biological activities that create vast possibilities for their use across multiple disciplines within clinical medicine [8].

Of particular scientific and clinical interest is the precise modification of chitosan to produce chitosan sulfate. The study of the sulfation reaction of chitosan has successfully established the fundamental regularities of this reaction from the theoretical standpoint of high-molecular-weight compounds. Detailed physicochemical characteristics of the resulting chitosan derivatives have been thoroughly documented at both the molecular and supramolecular structural levels. The synthesis and subsequent formation of chitosan sulfate have been rigorously analyzed in previous works through an array of high-precision identification experiments. These methodologies included infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy, X-ray diffraction analysis, high-resolution polarization microscopy, and the study of sorption capacities in aqueous environments [8].

Within this context, the scientific problem of creating domestic, highly effective, and accessible medicines remains exceptionally relevant today. Addressing this medical necessity, researchers at the Institute of Chemistry and Physics of Polymers of the Academy of Sciences of the Republic of Uzbekistan successfully developed a novel pharmaceutical formulation named Sulfoparin [8]. Sulfoparin is a specialized chitosan sulfate complex designed explicitly for its promising antisclerotic properties [2], [3], [6].

While preliminary evidence indicates high efficacy in therapeutic models, there remains limited information in the available scientific literature regarding the comprehensive low-toxicity profile of Sulfoparin. Specifically, detailed assessments of its long-term application in laboratory

animal models are sparse. There is an essential need for robust empirical data concerning the exact nature and severity of any potential damaging effects Sulfoparin might exert on the physiological systems of experimental animals during prolonged administration. Establishing a definitively safe toxicological profile is an absolute prerequisite before a novel drug can advance to widespread clinical application.

Consequently, the primary purpose of this research work is to rigorously investigate and document the chronic toxicity profile of the newly developed anti-atherosclerotic drug Sulfoparin.

**LITERATURE REVIEW**

The burden of cardiovascular and age-related metabolic diseases continues to drive innovation in the discovery of natural and semi-synthetic therapeutic agents. Atherosclerosis, characterized by the progressive accumulation of lipid plaques, endothelial dysfunction, and chronic vascular inflammation, requires long-term pharmacological management. Over the past decade, a substantial body of literature has recognized marine-derived polysaccharides, particularly chitin and its derivatives, as highly promising candidates for mitigating such pathological processes.

Chitosan is a linear, natural, nontoxic cationic polysaccharide that presents unique advantages in biomedical applications compared to neutral or negatively charged polymers. A growing consensus in the literature highlights the significant potential of chitosan and its modifications in the prevention and treatment of various age-related diseases, including cardiovascular dysfunctions and hypercholesterolemia. Crucially, the hypocholesterolemic properties inherent to chitosan have been shown to directly decrease the systemic risk of developing atherosclerosis. Furthermore, its ability to lower serum total cholesterol and low-density lipoprotein (LDL) cholesterol levels, while simultaneously preventing their detrimental oxidative modification, underscores its value as an anti-atherosclerotic base compound.

However, native chitosan exhibits limited solubility at physiological pH, prompting researchers to develop highly functionalized derivatives. The structural modification of chitosan via sulfation generates chitosan sulfate, an analog to the naturally occurring anticoagulant heparin. Sulfated marine polysaccharides have been widely documented for their favorable interactions within the cardiovascular system. Recent scientific investigations have indicated that marine natural products and specifically sulfated polysaccharides possess potent capabilities to suppress vascular inflammation. It has been demonstrated that when chitosan sulfate is effectively localized within the perivascular environment, it can promote remedial angiogenesis and successfully restrain the initial cascades of atherosclerotic inflammation.

In the specific context of Sulfoparin, domestic researchers have made significant strides. As previously mentioned, Sulfoparin was synthesized by the Institute of Chemistry and Physics of Polymers to leverage these precise antisclerotic mechanisms [8]. Preliminary localized studies have investigated the drug's fundamental mechanisms, demonstrating its capability to induce significant hypocholesterolemic action [2]. Furthermore, targeted research has highlighted Sulfoparin’s efficacy in the active correction of endothelial dysfunction in experimental rabbit models afflicted with induced hypercholesterolemia [3]. Additional studies by Kutlikova have delineated Sulfoparin's positive modulatory effect on the nitric oxide system, which is a vital component of vascular relaxation and endothelial health [6].

Despite these clear pharmacological benefits, regulatory frameworks strictly mandate comprehensive toxicological profiling [1], [4], [9]. Long-term antisclerotic therapies demand impeccably high safety margins, given that patients may consume these medications continuously over a span of years or decades. Chronic toxicity assessments must evaluate cumulative toxic properties, target organ vulnerability, and subtle metabolic disturbances that acute toxicity models

inherently fail to detect. While acute toxicity evaluations may classify a compound as generally safe, only a dedicated chronic toxicity model can definitively validate the biocompatibility of Sulfoparin for chronic human application. Thus, bridging this gap in the literature regarding Sulfoparin's long-term biosafety constitutes the core focus of the current investigation.

## MATERIAL AND RESEARCH METHODS

The primary object of this experimental research was the drug Sulfoparin, which is explicitly intended for clinical use as an anti-atherosclerotic agent. The specific formulation of Sulfoparin utilized throughout this study was synthesized and provided by the Institute of Chemistry and Physics of Polymers of the Academy of Sciences of the Republic of Uzbekistan.

The chronic toxicological studies of Sulfoparin were systematically conducted on a population of 80 healthy white laboratory rats of both sexes. At the onset of the experiment, the body weight of the experimental animals ranged from 110 to 120 grams. To simulate a continuous therapeutic regimen, the animals received freshly prepared aqueous solutions of the drug administered intragastrically on a daily basis over an uninterrupted period of 3 months. This duration and methodology strictly adhered to the established standard guidelines for the experimental preclinical study of new pharmacological substances [4], [9].

To evaluate a spectrum of dosage impacts, the experimental animals were randomly divided into four distinct groups, with exactly 20 animals assigned to each group. The first group of animals received Sulfoparin at a high dosage of 500 mg/kg of body weight. The second group received Sulfoparin at an intermediate dosage of 100.0 mg/kg. The third group received a relatively low therapeutic dosage of 25 mg/kg. The fourth group served as the negative control group and received an equivalent volume of standard physiological saline solution via the same intragastric route.

The comprehensive indicators used to assess systemic toxicity included continuous daily observations of animal behavior, calculations of the overall survival rate, precise recording of the time of death (if any occurred), and meticulous examinations for the appearance of any physical symptoms of intoxication. Furthermore, the dynamics of the animals' body weight were recorded at regular intervals to track generalized metabolic health.

Upon the conclusion of the 3-month experimental administration period, extensive laboratory investigations were performed. The content of hemoglobin, erythrocytes (red blood cells), and leukocytes (white blood cells) in the peripheral blood was studied utilizing classical, generally accepted hematological methodologies. The biochemical analysis focused on the activity of critical hepatic and metabolic enzymes in the blood serum. Specifically, the activity of alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the antioxidant enzyme catalase were quantified using standardized commercial biotests procured from Lachema (Czech Republic).

Kidney function was rigorously evaluated by monitoring diuresis levels after four hours. The state of nitrogen metabolism, for which the primary criterion is the concentration of urea in the blood, was also thoroughly quantified. Additionally, the presence of glucose and total protein in the urine, alongside urine acidity (pH), was measured to detect any potential tubular or glomerular nephrotoxicity.

Following the conclusion of the functional tests, a full macroscopic pathological examination was performed during the autopsy of the animals. Light-optical microscopic examinations of the internal organs and brain tissues of all experimental and control groups were conducted to identify any subtle cellular deviations. All obtained numerical research results were subjected to standard statistical processing to determine the significance of variance between the experimental groups and the control baseline.

## RESULTS

The fundamental parameters defining the chronic toxicometry of Sulfoparin had not been previously established in a long-term model, which served as the core rationale for these investigations. Throughout the entire 3-month duration of the experiment, the general physiological condition of the experimental animals was observed to be completely undisturbed. No overt or subtle symptoms of systemic intoxication were detected in any of the dosage cohorts, and an absolute survival rate was maintained with zero occurrences of animal death. Furthermore, detailed physical examinations revealed no localized pathological changes on the dermal layers. There were no noted areas of focal baldness (alopecia) or ulcerations. The animals maintained a neat appearance; their coats remained smooth and distinctly shiny. They demonstrated a normal, healthy appetite, consistently readily consuming their provided food, remained physically active, and continuously responded adequately to varied external stimuli.

A primary indicator of generalized toxic stress in animal models is a deviation in expected body weight gain. As explicitly demonstrated by the data presented in Table 1, no statistically significant lag in the dynamics of body weight gain was established in any of the experimental animals when compared directly to the control group animals throughout the three months or the subsequent recovery period.

**Table 1. Dynamics of body weight of white rats after intragastric repeated administration of Sulfoparin in various doses for 3 months (in grams)**

| Name of Animal Groups | Statistical Indicators | Background | 1 Month | 2 Months | 3 Months | Recovery Period |
|---|---|---|---|---|---|---|
| **Control** | M±m | 140±4.1 | 148±4.3 | 157±4.2 | 165±7.6 | 173±7.9 |
| **Sulfoparin, 500 mg/kg** | M±mR | 142±6.6 | 151±10.1 | 159±7.8 | 164±6.1 | 171±6.5 |
| **Sulfoparin, 100 mg/kg** | M±mR | 141±9.9 | 149±12.2 | 155±4.8 | 167±5.8 | 170±7.2 |
| **Sulfoparin, 25.0 mg/kg** | M±mR | 143±7.9 | 147±5.6 | 158±7.2 | 163±4.9 | 174±7.4 |

Note: P > 0.05 for all experimental indices relative to the control baseline.

In addition to gross physical metrics, the internal hematopoietic stability of the subjects was assessed. The dynamics of the content of essential cellular components—specifically hemoglobin, erythrocytes, and leukocytes—in the peripheral blood were meticulously studied. The comprehensive analysis did not reveal any statistically significant differences in the blood profile of the animals in the experimental groups when compared to the corresponding control data. The stability of these metrics, detailed in Table 2, suggests an absence of bone marrow suppression or drug-induced inflammatory leukocytosis.

**Table 2. The content of hemoglobin, erythrocytes, and leukocytes in the peripheral blood of white rats after intragastric administration of Sulfoparin for 3 months**

| Name of Animal Groups | Statistical Indicators | Hemoglobin content (g/l) | RBC content | Leukocyte count (10^9/l) |
|---|---|---|---|---|

| | | | (10^12/l) | |
|---|---|---|---|---|
| **Control** | M±m | 127.0±5.8 | 4.90±0.19 | 8.09±0.30 |
| **Sulfoparin, 500 mg/kg** | M±mR | 130.0±5.5 | 4.87±0.25 | 8.16±0.31 |
| **Sulfoparin, 100 mg/kg** | M±mR | 129.0±3.6 | 4.85±0.44 | 8.17±0.17 |
| **Sulfoparin, 25.0 mg/kg** | M±mR | 131.0±9.1 | 4.82±0.40 | 8.14±0.32 |

Note: P > 0.05 for all experimental indices relative to the control baseline.

Hepatotoxicity and general metabolic disruption were evaluated via serum biochemistry. Data derived from the investigation of the biochemical parameters of the blood serum of both experimental and control animals are systematically presented in Table 3. The enzymatic data obtained from the experimental animals regarding the activity of alkaline phosphatase, AST, ALT, and catalase in the circulating blood did not deviate from the control values. As the data in Table 3 clearly demonstrate, statistically significant differences in the activity of the studied hepatic and antioxidant enzymes in the blood of the experimental rats, compared to the control group results, have not been established. This confirms normal liver function and a lack of oxidative stress induction.

**Table 3. Biochemical parameters of blood serum of white rats after intragastric administration of Sulfoparin for 3 months**

| **Name of Animal Groups** | **Statistical Indicators** | **Alkaline phosphatase activity (mol/l.h)** | **AST activity (mmol/l.h)** | **ALT activity (mmol/l.h)** | **Catalase activity (Mcat/l)** |
|---|---|---|---|---|---|
| **Control** | M±m | 0.37±0.01 | 0.31±0.02 | 0.23±0.025 | 23.01±2.14 |
| **Sulfoparin, 500 mg/kg** | M±mR | 0.34±0.01 | 0.33±0.04 | 0.21±0.019 | 20.05±1.36 |
| **Sulfoparin, 100 mg/kg** | M±mR | 0.35±0.01 | 0.35±0.06 | 0.19±0.017 | 22.04±1.49 |
| **Sulfoparin, 25.0 mg/kg** | M±mR | 0.38±0.02 | 0.30±0.04 | 0.22±0.013 | 21.04±1.16 |

Note: P > 0.05 for all experimental indices relative to the control baseline.

Given that the kidneys are primary organs for drug filtration and excretion, renal parameters were strictly monitored. The results of the studies focusing on the functional state of the kidneys of the rats, exactly 3 months after the initiation of the continuous administration of Sulfoparin in doses of 500.0 mg/kg, 100.0 mg/kg, and 25.0 mg/kg, provided definitive evidence of safety. Specifically, the diuresis output of the experimental rats did not differ statistically from that of the control rats. The state of nitrogen metabolism, the central criterion of which is the urea content in the blood, exhibited absolutely no deviations from the control baseline. Furthermore, crucial indicators of glomerular damage, such as the presence of glucose and protein, were entirely absent in the urine of both the experimental and control groups throughout the entire duration of the experiment. The overall acidity of the urine across all studied groups remained stable during the entire experiment, maintaining a neutral pH of 7.0, as outlined in Table 4.

**Table 4. Some indicators of kidney function in experimental and control rats, 3 months after administration of Sulfoparin in varied doses**

| Groups of Animals | Drug Dose (mg/kg) | Diuresis after 4 hours (%) | Blood Urea (mmol/l) |
|---|---|---|---|
| **Control** | 0 | 91±1.6 | 4.6±0.13 |
| **Sulfoparin** | 500.0 | 90±1.3 | 4.7±0.12 |
| **Sulfoparin** | 100.0 | 89±1.88 | 4.8±0.13 |
| **Sulfoparin** | 25.0 | 89±1.45 | 4.5±0.31 |

Note: $P > 0.05$ relative to the control group.

Microscopic evaluation of urine sediment did not reveal any pathological changes, further supporting the biochemical data. The cumulative results firmly allow us to conclude that the drug does not exert any underlying toxic effect on essential kidney function. Thus, it was robustly established that Sulfoparin does not possess a toxic effect at any of the extensive doses studied.

Following the in-vivo evaluations, a generalized post-mortem examination of the bodies of the white rats after the 3-month intragastric administration regimen demonstrated a complete absence of macroscopically recognizable abnormalities when compared directly to the anatomy of animals in the control group. All dissected animals maintained a regular physique, a healthy outward appearance, and shiny coats, with no cutaneous lesions identified. The visible mucous membranes were noted to be moist, pale pink, and entirely smooth in appearance.

The individual mass measurements of the internal organs of the white rats, taken during the macroscopic pathological examination during the autopsy phase following the completion of the experiment, verified that no hypertrophic or atrophic changes had occurred in the experimental animals. Most importantly, high-resolution light-optical microscopic examinations of cross-sections from the vital internal organs and the brain of all experimental groups definitively showed a total absence of structural or cellular deviations from the normal physiological baseline.

## DISCUSSION

The results of the present study provide compelling evidence regarding the exceptional safety profile of the anti-atherosclerotic agent Sulfoparin. In toxicological pharmacology, the chronic administration model is the gold standard for predicting the long-term safety of a drug intended for continuous human use. Given that atherosclerosis is a chronic, progressive condition requiring sustained therapeutic intervention, any viable antisclerotic pharmaceutical must demonstrate near-total biological tolerance over extended periods.

The absence of any deviation in body weight dynamics across all three dosage levels—including the extremely high 500 mg/kg group—strongly indicates that Sulfoparin does not interfere with baseline metabolic processes, nutrient absorption, or basic caloric utilization. Furthermore, the stability of the hematological parameters (hemoglobin, erythrocytes, and leukocytes) suggests that the drug does not cross-react with the bone marrow or induce systemic immune-inflammatory cascades.

The hepatic and renal systems are typically the most vulnerable to xenobiotic toxicity due to their primary roles in drug metabolism and excretion. Our biochemical analysis verified that essential transaminases (AST, ALT) and alkaline phosphatase remained within strict physiological norms. Elevation in these enzymes is universally recognized as a primary indicator of hepatocellular necrosis or biliary dysfunction [1]. The lack of fluctuation in these values confirms that Sulfoparin is processed by the liver without causing cellular stress. Similarly, the consistent measurement of blood urea and optimal diuresis outputs prove that the renal filtration apparatus, specifically the delicate glomeruli and tubules, remains unharmed by the drug or its metabolites.

This physiological stability was perfectly mirrored by the macroscopic and light-optical microscopic histological findings, which showed an absolute absence of dystrophic or necrobiotic lesions.

These findings align seamlessly with recent global literature surrounding chitosan-based biopolymers. As noted by leading researchers, chitosan and its sulfated derivatives are celebrated for their high degree of biocompatibility and lack of inherent toxicity. The structural similarity between chitosan sulfate and naturally occurring glycosaminoglycans like heparin likely contributes to its seamless integration into mammalian physiology without triggering foreign-body toxic responses. The successful demonstration of Sulfoparin's safety effectively paves the way for further advanced clinical trials, securely establishing its viability as a long-term therapeutic modality for hypercholesterolemia and endothelial dysfunction.

**CONCLUSIONS**

Based on the extensive preclinical data gathered during this three-month continuous evaluation, the following definitive conclusions have been drawn:

With long-term, chronic intragastric administration, the therapeutic agent Sulfoparin does not negatively affect the outward behavior, survival rate, or the baseline dynamics of body weight gain in experimental animal models.

The drug does not exert any observable toxic effects on critical hematological parameters, foundational kidney and liver organ functions, nor does it present any negative structural effects on the localized morphology of internal organs and neural tissues.

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