UDK 615.9:616-092.4

Кутликова Г.М.

Заведующая кафедрой пропедевтики внутренних болезней,

Андижанский филиал Кокандского университета

ORCID: 0000-0001-5196-7762

Ниязов З.М.

Доцент кафедры пропедевтики внутренних болезней,

Андижанский филиал Кокандского университета

ORCID: 0009-0000-0328-6997

Орифджонов Р.Р.

Доцент кафедры пропедевтики внутренних болезней,

Андижанский филиал Кокандского университета

ORCID: 0009-0003-8933-1681

Исмоилов Д.М.

Преподаватель кафедры пропедевтики внутренних болезней.

Андижанский филиал Кокандского университета

ORCID: 0009-0005-3054-6842

ДЕКЛИНИЧЕСКАЯ ТОКСИКОЛОГИЯ СУЛЬФОПАРИНА ИЗ ХИТОЗАНА, ПОЛУЧЕННОГО ИЗ ШЕЛКОВИЦ

Аннотация Введение: Оценка безопасности препаратов сульфатированного хитозана требует наличия данных, специфичных для каждой рецептуры, и надежной документации о дозе, способе введения и побочных эффектах. Цель: Обобщить данные о токсикологических наблюдениях при остром, повторном введении и развитии сульфопарина, представленные в диссертации, выявив при этом несоответствия, ограничивающие интерпретацию безопасности. Методы: Сводные данные и описательные наблюдения были извлечены из главы по

токсикологии диссертации. Количество случаев острой смертности было арифметически сопоставлено с размерами групп. Масса тела при повторном введении, показатели функции почек и некоторые биохимические показатели, а также масса органов были оценены описательно. Новые критерии значимости или нормативные классификации безопасности не были разработаны. Результаты: Количество случаев острой смертности составило 0/10, 2/10, 4/10, 6/10 и 10/10 при номинальных дозах 1000, 1500, 2000, 2500 и 3000 мг/кг. Указанная медианная летальная доза составила 2150 мг/кг; способ введения препарата не совпадает между методами и заголовком таблицы. В трехмесячном эксперименте на крысах конечная масса тела составила 159,5 г в группе с номинальной дозой 500 мг/кг и 185,5 г в контрольной группе, разница составляет 14,0%. Показатели активности аспартатаминотрансферазы и массы нескольких органов также численно различались, несмотря на общее утверждение источника об отсутствии токсичности. Сводные показатели функции почек были близки в разных группах. Наблюдения за развитием показали аналогичную выживаемость эмбрионов, но содержали противоречивые данные о дозах и неполные данные по пометам. Выводы: Данные документируют острую токсичность при достаточно высоких номинальных дозах и предоставляют предварительные наблюдения при многократном введении. Они не устанавливают общую безопасность, отсутствие репродуктивной токсичности или надежный уровень отсутствия наблюдаемых неблагоприятных эффектов. Для подтверждения возможности применения препарата в клиническую практику необходимы оригинальные записи и, при необходимости, подтверждающие исследования.

Ключевые слова: сульфопарин; сульфатированный хитозан; доклиническая токсикология; токсичность при многократном введении; токсичность для развития; проверка данных

Qutlikova G.M.
Head of the Department of Propaedeutics of Internal Medicine.,
Andijan Branch of Kokand University
ORCID: 0000-0001-5196-7762

Niyazov Z.M.
Associate Professor, Department of Propaedeutics of Internal Medicine.,
Andijan Branch of Kokand University
ORCID: 0009-0000-0328-6997

Orifdjonov R.R.
Associate Professor, Department of Propaedeutics of Internal Medicine.,
Andijan Branch of Kokand University
ORCID: 0009-0003-8933-1681

Ismoilov D.M.
Lecturer, Department of Propaedeutics of Internal Medicine.
Andijan Branch of Kokand University
ORCID: 0009-0005-3054-6842

PRECLINICAL TOXICOLOGY OF SULFOPARIN FROM SILKWORM DERIVED CHITOSAN

Abstract

Background: Safety assessment of sulfated chitosan preparations requires formulation-specific evidence and reliable documentation of dose, route and adverse outcomes.

Objective: To summarize the acute, repeated-dose and developmental toxicology observations reported for sulfoparin in a supplied dissertation, while identifying inconsistencies that limit safety interpretation.

Methods: Aggregate data and narrative observations were extracted from the dissertation's toxicology chapter. Acute mortality counts were reconciled arithmetically with group sizes. Repeated-dose body weights, renal measurements and selected biochemical and organ-weight findings were evaluated descriptively. No new significance tests or regulatory safety classifications were generated.

Results: Acute mortality counts were 0/10, 2/10, 4/10, 6/10 and 10/10 at nominal doses of 1000, 1500, 2000, 2500 and 3000 mg/kg. The reported median lethal dose was 2150 mg/kg; the administration route is inconsistent between the methods and a table

heading. In the three-month rat experiment, final body weight was 159.5 g in the nominal 500 mg/kg group and 185.5 g in controls, a 14.0% difference. Aspartate aminotransferase and several absolute organ weights also differed numerically despite the source's general statement of no toxicity. Renal summary measurements were close across groups. Developmental observations suggested similar embryo survival but had irreconcilable dose labels and incomplete litter-level reporting.

Conclusions: The dataset documents acute toxicity at sufficiently high nominal doses and provides preliminary repeated-dose observations. It does not establish general safety, absence of reproductive toxicity or a reliable no-observed-adverse-effect level. Original records and, where necessary, confirmatory studies are required before translational claims can be made.

Keywords: sulfoparin; sulfated chitosan; preclinical toxicology; repeated-dose toxicity; developmental toxicity; data verification

Introduction

The biological activity of sulfated chitosan depends on the characteristics of the individual preparation. Experimental anticoagulant studies have linked activity to structural features and interactions with coagulation regulators [1]. Consequently, favorable findings for one derivative cannot establish the safety of a different preparation, and natural origin is not sufficient evidence of tolerability.

The supplied dissertation describes sulfoparin, a locally produced sulfated chitosan derived from silkworm material, and reports acute, repeated-dose, local-tolerance and reproductive investigations [2]. The source concludes broadly that the preparation lacks toxic, cumulative, sensitizing and reproductive effects. However, the underlying tables include mortality at high doses, differences in some repeated-dose endpoints and inconsistent exposure descriptions. These observations require a more restricted interpretation.

Modern reporting frameworks help distinguish absence of observed effects from sufficient evidence of safety. ARRIVE 2.0 emphasizes transparent animal-study reporting [3], while OECD Test Guidelines 408 and 414 provide reference frameworks for repeated-dose and prenatal developmental studies [4,5]. They are used here as reporting benchmarks; the historical experiments are not retrospectively described as compliant with those guidelines.

This manuscript aims to present the recoverable toxicology evidence, preserve the distinction between reported observations and verified conclusions, and identify

which findings require primary-record review. It concerns safety endpoints in separate animal experiments and does not reuse the rabbit efficacy outcomes as toxicological evidence.

Materials and methods

Source and scope

The analysis used the methods chapter and toxicology Tables 5.1–5.12 of the supplied English dissertation [2]. No animal-level dataset, pathology slides, complete laboratory reports or contemporaneous study protocols were available. The original programme lists 80 mice, 212 rats, 20 guinea pigs and 27 rabbits for toxicology, but subgroup descriptions do not consistently reconcile with those totals. Species totals are therefore not used as endpoint denominators.

Test material and exposure documentation

Sulfoparin is described as an aqueous preparation of sulfated chitosan developed in Uzbekistan. Batch-specific composition, molecular-weight distribution, degree of sulfation, residual contaminants, endotoxin content, stability and delivered concentration are not adequately documented in the supplied text. These omissions restrict interpretation because nominal mass dose alone does not define the exposure to a chemically heterogeneous material.

Acute toxicity experiment

The methods describe 60 mice of both sexes, weighing 18–22 g, allocated to five active-dose groups and a control group. Each active-dose group contained ten animals and received a nominal dose of 1000, 1500, 2000, 2500 or 3000 mg/kg. The methods describe intragastric administration, with larger doses divided into two administrations one hour apart, whereas Table 5.1 labels the route as intramuscular. The route and split-dose allocation remain unresolved.

Clinical observations included appearance, behavior, food intake and respiratory changes. Observation is described as hourly on the first day and subsequently for two to three weeks, while the mortality table also contains an ambiguously labeled column for later deaths. The dissertation reports calculation of a median lethal dose using historical statistical procedures. This estimate is reproduced as a source result and has not been independently fitted.

Repeated-dose rat experiment

The methods describe 80 rats weighing 110–120 g, with 20 animals in each of four groups treated daily for three months. The lower and upper nominal doses were 25 and 500 mg/kg. The intermediate dose is 100 mg/kg in the methods and 250 mg/kg in the results tables. Table captions identify n = 6 for several endpoints without explaining how these animals were selected from the groups of 20.

Recorded outcomes included general condition, survival, body weight, hematology, serum biochemistry, urine observations, absolute organ weights and descriptive histology. Intragastric administration is described for the repeated-dose experiment, but the renal-table caption refers to intravenous treatment. Results are therefore organized by the printed dose labels without treating the administration route or exposure identity as confirmed.

Developmental and local-tolerance observations

The developmental study describes three groups of 20 pregnant rats, with daily exposure during gestation. The methods list 0.5 and 5.0 mg/kg, whereas the principal developmental tables label 25 and 250 mg/kg; another caption refers to 100 mg/kg. These discrepancies prevent assignment of a verified dose to the developmental outcomes.

The source also describes skin application, ocular observations, sensitization tests and an escalating-dose experiment intended to assess cumulative effects. Because sample totals, challenge units and outcome definitions are inconsistently reported, these components were summarized narratively rather than used to establish quantitative safety margins.

Data reconciliation and analysis

Acute mortality percentages were calculated from reported total deaths divided by the corresponding group size. Other numerical differences were calculated from the displayed group means. Printed ± quantities were retained without assuming that all tables consistently report standard errors. No missing values were imputed, no malformed hematology entries were reconstructed and no significance tests were performed on the aggregate summaries.

The analysis distinguishes three levels of evidence: a number printed in a table, an interpretation stated in the source, and a conclusion supportable from the available

documentation. Where these disagree, the discrepancy is retained. The evaluation does not assign a regulatory hazard category or a no-observed-adverse-effect level.

Ethics and welfare reporting

An ethics committee name, approval identifier and approval date were not available in the supplied text. The source describes terminal procedures but lacks sufficient detail to assess their authorization and implementation. The original investigators must provide contemporaneous approval, humane endpoints, monitoring and procedural records before submission [3].

Results

Acute mortality and clinical observations

Total deaths increased with nominal dose: zero at 1000 mg/kg, two at 1500 mg/kg, four at 2000 mg/kg, six at 2500 mg/kg and ten at 3000 mg/kg (Table 1). The first dose row prints a mortality percentage of 10% despite zero recorded deaths; arithmetic from the death count yields 0%. This correction is explicitly identified and should be checked against individual animal records.

The narrative describes reduced activity, reduced food intake, an unkempt appearance and respiratory cessation in affected animals. The source-reported LD50 was 2150 mg/kg, with endpoints printed as 1874.6 and 2425.4 mg/kg. Their confidence level is not clearly specified, so they are not labeled a verified 95% confidence interval. Neither the LD50 route nor the timing of all deaths can be established reliably from the translated document.

Table 1 Acute mortality by nominal sulfoparin dose

Dose mg/kg	Animals	Total deaths	Calculated mortality
1000	10	0	0%†
1500	10	2	20%
2000	10	4	40%
2500	10	6	60%
3000	10	10	100%

Source Table 5.1. Percentages are calculated from the reported total death counts. †The source prints 10% despite zero deaths at 1000 mg/kg. Route is intragastric in the methods but intramuscular in the table heading; final observation timing is also unresolved. No control mortality row is available in this table.

Repeated-dose body weight and biochemical findings

The source states that repeated treatment produced no deaths or overt intoxication and no statistically significant delay in weight gain. Nevertheless, the final mean body weight in the nominal 500 mg/kg group was 159.5 ± 4.1 g, compared with 185.5 ± 2.2 g in controls (Table 2). This is a 14.0% lower final mean. Descriptive mean gain from the respective baseline means was 44.7 g versus 68.0 g, or 34.3% less gain. These calculations do not establish significance or adversity, but they require investigation before a no-effect conclusion is accepted.

Table 2 Baseline and final body weights in the repeated dose study

Nominal group	Baseline g	Month 3 g	Mean gain g†
25 mg/kg	118.0 ± 2.2	193.3 ± 2.4	75.3
250 mg/kg‡	119.8 ± 2.4	177.0 ± 4.1	57.2
500 mg/kg	114.8 ± 5.8	159.5 ± 4.1	44.7
Control	117.5 ± 2.4	185.5 ± 2.2	68.0

Source Table 5.4, caption n = 6; the methods describe n = 20 per group. Values retain the source ± notation. †Gain is the difference between displayed group means, not a new individual-level analysis. ‡The intermediate dose is 100 mg/kg in the methods and 250 mg/kg in the tables. The malformed month-one entry is excluded rather than imputed.

Selected biochemical entries raise similar questions. Aspartate aminotransferase was reported as 83.3 ± 0.23 U/L in the 500 mg/kg group versus 60.0 ± 2.23 U/L in controls, a 38.8% difference. Alanine aminotransferase in the intermediate-dose group was 15.57 ± 1.19 U/L versus 35.7 ± 4.15 U/L in controls. These entries conflict with the narrative impression of uniformly similar biochemistry, although the direction and clinical meaning cannot be resolved without the original assay reports.

Several hematology cells have missing separators or implausible unit labels and cannot be reliably interpreted. In the biochemical table, some HDL cholesterol values exceed the corresponding total cholesterol values. Those lipid entries are not suitable

for a safety conclusion without verification. The month-one body weight of 14.5 g in the lower-dose group is also not silently changed to a presumed alternative value.

Renal and organ observations

Blood urea means ranged from 4.5 to 4.8 mmol/L across treated groups, compared with 4.6 mmol/L in controls (Table 3). The reported four-hour diuresis measure ranged from 89% to 90% in treated animals versus 91% in controls. The denominator underlying this percentage is not specified. The source reports no urine glucose or protein and a urine pH of 7.0 in all groups.

Table 3 Selected renal measurements after repeated treatment

Nominal group	Four hour diuresis %†	Blood urea mmol/L
Control	91 ± 1.6	4.6 ± 0.13
25 mg/kg	89 ± 1.45	4.5 ± 0.31
250 mg/kg‡	89 ± 1.88	4.8 ± 0.13
500 mg/kg	90 ± 1.3	4.7 ± 0.12

Source Table 5.7. †The denominator for percentage diuresis is unspecified. ‡The intermediate dose and administration route are inconsistent across the source. Values retain the printed ± notation; endpoint n and dispersion definition require confirmation. Similar group means do not establish renal safety.

Descriptive histology was interpreted in the dissertation as showing no major destructive or inflammatory lesions. However, blinded scoring, individual lesion incidence and complete histological documentation are not available. Absolute spleen weight in the intermediate-dose group was 1.30 ± 0.07 g versus 0.75 ± 0.06 g in controls, and kidney weight in the lower-dose group was 0.85 ± 0.06 g versus 0.50 ± 0.09 g. These differences require assessment using body-weight-adjusted organ measurements and pathology records; they cannot be dismissed solely on the basis of a general narrative statement.

Developmental and additional observations

The principal developmental table reports embryo survival of 93.7% in controls and 93.8% and 93.5% in the two treated groups. Fetal weights were 2.55, 2.62 and 2.48 g, respectively. These descriptive similarities are limited by the unresolved doses and

the absence of litter-level data, maternal exposure information and a clear accounting of dams assigned to terminal fetal assessment versus postnatal observation.

Skin and ocular experiments were described as showing no observed local reaction under the tested conditions. Sensitization experiments were also interpreted as negative, but challenge-dose units and animal counts are inconsistent. The escalating-dose table records deaths in later intervals; it does not, by itself, establish absence of pharmacokinetic accumulation. These components are preliminary observations rather than proof of universal absence of irritation, allergy or cumulative toxicity.

Discussion

The acute results demonstrate that sulfoparin was not devoid of toxicity across the nominal dose range tested. Mortality increased with dose, and the reported LD50 lies between dose levels associated with 40% and 60% mortality. That numerical coherence is useful, but route and observation-period discrepancies prevent a definitive route-specific acute-toxicity assessment.

The repeated-dose findings require more careful evaluation than the source's broad safety conclusion suggests. Lower final body weight, a higher AST mean and several organ-weight differences are potential signals requiring reconciliation. They are not sufficient to diagnose organ injury: transcription errors, assay issues, group imbalance or biological variation remain possible. Conversely, incomplete documentation is not a basis for declaring the findings harmless.

OECD Test Guideline 408 considers repeated-dose assessment across clinical observations, body weight, laboratory measurements and pathology [4]. The present source contains elements of those domains but lacks the coherent animal-level record needed to determine whether they agree. In particular, the unexplained reduction from 20 animals per group to n = 6 in several tables prevents assessment of missingness and selection bias. The current report therefore cannot establish a no-observed-adverse-effect level at 500 mg/kg or any other nominal dose.

Developmental interpretation is further restricted by conflicting exposure labels. A fetal outcome cannot support a dose-specific safety statement when the administered dose itself is uncertain. Litter structure also matters because fetuses from one dam share maternal exposure and cannot automatically be treated as independent replicates. OECD Test Guideline 414 provides a framework for recording maternal and developmental outcomes, but the available aggregate summaries do not demonstrate that framework's implementation [5].

Negative local-tolerance observations have a narrower meaning than absence of sensitization risk. They apply to the documented formulation, concentration, application conditions and observation period, insofar as those can be recovered. The present preparation may have anticoagulant properties, but the toxicology summaries do not provide a validated assessment of bleeding risk or interactions. Published mechanistic work on other chitosan polysulfates underscores the need to characterize the specific compound [1].

No margin between rabbit efficacy and rodent toxicity can be calculated responsibly from these reports. The efficacy doses are printed in μg/kg, toxicology doses in mg/kg, and administration routes vary or are unclear. Nominal-dose ratios across species do not replace pharmacokinetic exposure measurements. Such ratios would be particularly misleading here because dose identity is one of the unresolved issues.

The strongest contribution of this analysis is the distinction between apparently reassuring observations and a defensible safety conclusion. Renal summary measurements and qualitative histology may warrant further evaluation, while discordant body-weight and biochemical entries should guide primary-record retrieval. If those records cannot resolve the conflicts, appropriately designed confirmatory studies would be needed rather than stronger wording applied to the same uncertain summaries.

Conclusions

The dissertation provides preliminary toxicology observations for sulfoparin and documents dose-associated acute mortality. Internal inconsistencies and possible repeated-dose signals prevent a general conclusion of safety, a reliable no-observed-adverse-effect level or an assertion of absent developmental toxicity. Verification of exposure, endpoint data and pathology is necessary before the findings can support further translational development.

Declarations

Ethics approval: [Original committee name, approval identifier, date and procedural authorization required.]

Funding, competing interests and author contributions: [To be declared and approved by the actual investigators.]

Data availability: Only aggregate dissertation data were available for this draft. Original records, individual observations and pathology material must be addressed in the finalized statement.

Prior dissemination: Derived from the supplied dissertation. Authors must identify previous reports containing these toxicology experiments and disclose overlap.

Writing assistance: AI-assisted English drafting and document preparation were used; the authors must independently verify all scientific content and follow the selected journal's disclosure policy.

References

1. Suwan J., Zhang Z., Li B., Vongchan P., Meepowpan P., Zhang F., Mousa S. A., Mousa S., Premanode B., Kongtawelert P., Linhardt R. J. Sulfonation of papain-treated chitosan and its mechanism for anticoagulant activity // Carbohydrate Research. — 2009. — Vol. 344, No. 10. — P. 1190–1196. — DOI: 10.1016/j.carres.2009.04.016.
2. Percie du Sert N., Hurst V., Ahluwalia A., Alam S., Avey M. T., Baker M., Browne W. J., Clark A., Cuthill I. C., Dirnagl U., Emerson M., Garner P., Holgate S. T., Howells D. W., Karp N. A., Lazic S. E., Lidster K., MacCallum C. J., Macleod M., Pearl E. J., Petersen O. H., Rawle F., Reynolds P., Rooney K., Sena E. S., Silberberg S. D., Steckler T., Würbel H. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research // PLOS Biology. — 2020. — Vol. 18, No. 7. — Article e3000410. — DOI: 10.1371/journal.pbio.3000410.
3. Organisation for Economic Co-operation and Development (OECD). Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents // OECD Guidelines for the Testing of Chemicals. Section 4. — Paris : OECD Publishing, 2025. — DOI: 10.1787/9789264070707-en.
4. Organisation for Economic Co-operation and Development (OECD). Test No. 414: Prenatal Developmental Toxicity Study // OECD Guidelines for the Testing

of Chemicals. Section 4. — Paris : OECD Publishing, 2018. — DOI: 10.1787/9789264070820-en.

5. Qutlikova G. M., Yuldashev R. N., Xudayarova N. K., Vakhobov Kh. B., Ganieva N. A. Temporal changes in inflammatory and nitric oxide related biomarkers in rabbits with experimental hypercholesterolemia // Egyptian Medical Journal of the National Research Center. — [Year, volume, issue and page numbers to be verified]. — ISSN 2090-5386. — Available at: http://www.mjnrc.com/index.php/emjn (accessed: 02.11.2026).