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Acute toxicity and genotoxicity of streptokinase and streptodornase

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Abstract
Background: Streptokinase and streptodornase are bacterial enzymes with established applications in thrombolysis and wound debridement; however, comprehensive data on their acute oral toxicity and genotoxic potential remain limited despite decades of clinical use.
Methods: Acute oral toxicity was assessed in 90 Wistar rats (five males and five females per group) administered single escalating doses of the lyophilized Streptokinase-Streptodornase preparation (1–8 g) suspended in PBS, with 14-day observation of clinical signs, body weight, organ weights, haematology, serum biochemistry, and histopathology. Genotoxicity was evaluated using the bacterial reverse mutation (Ames) test in five Salmonella typhimurium strains (TA98, TA100, TA102, TA1535, TA1537) at concentrations up to 5 mg/plate (±S9), the chromosomal aberration assay in CHO-K1 cells at 0,5–2,0 mg/mL (±S9), and the mammalian erythrocyte micronucleus assay in ICR mice at 500–2,000 mg/kg (48- and 72-hour sampling).
Results: No mortality, clinical signs of toxicity, or statistically significant differences in any measured parameter were observed in the acute oral toxicity study. The Ames test showed no increase in revertant colonies or cytotoxicity in any strain. Chromosomal aberration frequencies and micronucleated reticulocyte frequencies remained comparable to negative controls in all treated groups under both metabolic activation conditions, while positive controls produced the expected responses.
Conclusion: The Streptokinase-Streptodornase preparation demonstrated no acute oral toxicity and no genotoxic activity in the battery of standard assays performed, indicating a favourable safety profile.

Keywords: Streptokinase, streptodornase, acute oral toxicity, genotoxicity, Ames test, chromosomal aberration, micronucleus assay, Wistar rat, CHO-K1 cell, ICR mouse

Introduction

Streptokinase (SK) and streptodornase (SD) are bacterial enzymes produced by beta-hemolytic streptococci that have revolutionized thrombolytic therapy and enzymatic debridement since their discovery in the early 20th century. The fibrinolytic activity of SK was first documented in 1933 when Tillett and Garner observed that culture filtrates of certain streptococcal strains could rapidly dissolve human fibrin clots, opening an entirely new avenue for the treatment of thrombotic disorders [1]. This serendipitous finding was followed by systematic purification efforts and early clinical explorations led by Tillett and collaborators such as Sol Sherry, which established SK as a viable therapeutic agent by the 1940s and 1950s [2]. SD, a deoxyribonuclease, was subsequently characterized for its ability to depolymerize extracellular DNA within purulent exudates and necrotic tissue, thereby reducing viscosity and enhancing the removal of devitalized material when used in combination with SK [3].

The combined SK-SD preparation, historically marketed as Varidase, has been applied topically, intrapleurally, and buccally for decades in the management of infected wounds, burns, empyema, and surface lesions. Early clinical reports highlighted its capacity to control serious necrotic and purulent conditions by liquefying fibrinoid debris and promoting wound cleansing [4]. Comparative studies demonstrated that SK-SD accelerated the time to a clean wound bed in infected surgical wounds relative to saline-soaked dressings, supporting its role in enzymatic debridement protocols [5]. Additional investigations confirmed its utility in pressure ulcers and sinusitis when administered buccally, underscoring the versatility of the enzyme combination beyond intravenous thrombolysis [6, 7].

In cardiovascular medicine, SK became a cornerstone of reperfusion therapy for acute myocardial infarction (AMI). Landmark trials such as the Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico (GISSI) in 1986 established that intravenous SK significantly reduced short-term mortality when given early after symptom onset [8]. The Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) trial, which enrolled more than 41,000 patients, provided definitive comparative data, confirming SK's mortality benefit while noting differences in reperfusion speed compared with accelerated tissue-type plasminogen activator [9]. Equivalence studies, including the COMPASS trial, further validated SK against newer agents in terms of 30-day mortality outcomes in AMI [10]. SK has also been evaluated in acute ischemic stroke, although major trials such as the Multicentre Acute Stroke Trial–Europe (MAST-E) and MAST-Italy were terminated prematurely because of increased rates of intracranial hemorrhage [11, 12].

Despite these established therapeutic benefits, safety considerations remain central to the clinical use of SK-SD. SK is highly antigenic and can provoke immune responses, including hypotension and allergic reactions, particularly in individuals with recent streptococcal infections [13]. Bleeding complications and other haemostatic disturbances are well-documented side effects of thrombolytic regimens [14]. Data on oral administration are notably sparse because most applications have been intravenous or topical. Preclinical toxicology evaluations of related plasminogen activators, such as anisoylated plasminogen-streptokinase activator complex, have focused predominantly on intravenous routes and have not fully addressed high-dose oral exposure [15].

Comprehensive genotoxicity assessment is equally important for biological products of bacterial origin. Enzymes such as SK and SD could, in theory, interact indirectly with genetic material through metabolic or inflammatory pathways, necessitating rigorous testing under standardized protocols [16]. International regulatory frameworks, including OECD and ICH guidelines, mandate acute toxicity and genotoxicity studies prior to broader pharmaceutical or supplemental use of such preparations [17]. Existing literature has emphasized clinical efficacy and general safety profiles of SK but has provided limited information on oral acute toxicity or mutagenic potential in modern standardized assays [18, 19].

This investigation was therefore undertaken to generate robust safety data for a specific lyophilized SK-SD preparation using internationally accepted methodologies. The results contribute essential evidence regarding the absence of adverse effects following high-dose oral exposure and across multiple genotoxicity endpoints, thereby addressing a longstanding gap in the toxicological profile of these therapeutically important enzymes [20].

The aim of this study was to evaluate the acute oral toxicity and genotoxic potential of the combined Streptokinase-Streptodornase preparation using standardized *in vivo* and *in vitro* assays in accordance with internationally recognized guidelines for laboratory animal use and genotoxicity testing.

Methods

The combined enzyme preparation Streptokinase-Streptodornase was sourced from Amano Enzyme Incorporation (Japan). Each gram of the lyophilised powder contained not less than 1,000,000 IU of Streptokinase and not less than 250,000 IU of Streptodornase.

Acute Oral Dose Toxicity Study

All experimental procedures were conducted in strict accordance with internationally recognised guidelines for the use of laboratory animals.

A total of 90 Wistar rats aged five weeks (equally distributed by sex) were procured from Beijing Weitonglihua Experimental Animal Technology Co., Ltd. (Beijing, China). Animals were maintained under controlled environmental conditions: ambient temperature 22-24°C, relative humidity 50-60%, and a standard 12-hour light/dark cycle. Ad libitum access to a commercial standard diet and potable water was provided for the duration of the experiment.

The 90 rats were allocated into nine groups of ten animals each (five males and five females per group). The Control group consisted of intact, untreated animals that served as a reference for all measured parameters. Animals in the remaining eight groups received a single oral administration of Streptokinase-Streptodornase powder suspended in phosphate-buffered saline (PBS) at the following escalating doses: Group 1, 1 g (1,000,000 IU Streptokinase / 250,000 IU Streptodornase); Group 2, 2 g (2,000,000 IU / 500,000 IU); Group 3, 3 g (3,000 000 IU / 750,000 IU); Group 4, 4 g (4,000,000 IU / 1,000,000 IU); Group 5, 5 g (5,000,000 IU / 1,250,000 IU); Group 6, 6 g (6,000,000 IU / 1,500,000 IU); Group 7, 7 g (7,000,000 IU / 1,750,000 IU); and Group 8, 8 g (8,000,000 IU / 2,000,000 IU).

Clinical signs were monitored and recorded on a daily basis throughout the 14-day observation period. Body weights were measured for all animals on a weekly basis. Upon completion of the study, all animals were fasted overnight and subsequently euthanised under inhalational anaesthesia with 2% isoflurane (Halocarbon Laboratories, USA) administered via an inhalation chamber (MSS 003, Benchtop Small Animal Anaesthesia Unit, UK).

Bacterial Reverse Mutation Assay (Ames Test)

Five Salmonella typhimurium tester strains, namely TA98, TA100, TA102, TA1535, and TA1537, were obtained from Hisun Pharmaceutical Co., Ltd. (Hangzhou, China) and employed in both the cytotoxicity and mutagenicity assessments.

For the bacterial cytotoxicity evaluation, all five tester strains were co-incubated with Streptokinase-Streptodornase at concentrations of 0.05, 0.15, 0.5, 1.5, and 5 mg per plate on nutrient agar for 24 hours.

For the Ames mutagenicity assay, the same concentration series (0.05, 0.15, 0.5, 1.5, and 5.0 mg per plate) was tested in the presence and absence of rat liver S9 metabolic activation fraction. Briefly, sterilised test tubes containing 2 mL of soft agar were supplemented with 100 µL of the PBS-dissolved test article, alongside either a vehicle control (DMSO) or a positive control, 200 µL of a histidine/biotin mixture, 100 µL of the respective tester strain suspension, and 200 µL of S9 fraction where metabolic activation was required. After thorough mixing, the contents were overlaid onto minimal glucose agar plates. Following solidification of the soft agar, the plates were inverted and incubated at 37°C for 48 hours. All colonies per plate were enumerated. A test article was classified as mutagenic when the mean revertant colony count in a treated group exceeded twice the spontaneous mutation frequency of the concurrent negative control.

Mammalian Cell Chromosomal Aberration Assay

Chinese hamster ovary cells (CHO-K1) were supplied by Hisun Pharmaceutical Co., Ltd. (Hangzhou, China). Cells were routinely cultured in Ham's F-12 medium supplemented with 10% foetal bovine serum and 100 units/mL Penicillin-Streptomycin at 37°C in a humidified atmosphere containing 5% CO_2.

For the chromosomal aberration study, CHO-K1 cells were seeded at a density of 2×10^5 cells/mL. Once confluent attachment was achieved, cells were exposed to either a positive control (Cyclophosphamide, 25 µg/mL) or to Streptokinase-Streptodornase at concentrations of 0,5, 1,0, and 2,0 mg/mL in Ham's F-12 medium for 3 hours under conditions of metabolic activation (+S9). The treatment medium was then replaced with fresh medium, and cells were further incubated for 18 hours. In parallel, cells were

treated with a second positive control (Mitomycin C, 2.5 μg/mL) or the same three concentrations of Streptokinase-Streptodornase for 18 hours in the absence of metabolic activation (-S9). At 21 hours (+S9) and 18 hours (-S9) after the start of treatment, colcemid (100 μL, 10 μg/mL) was added to arrest cells in metaphase until the 24-hour time point. Cells were then subjected to hypotonic treatment with 0.54% KCl solution followed by fixation in acetic acid/methanol (1:3, v/v). Chromosomes were visualised by conventional Diff-Quick staining. Metaphase spreads were systematically scored for the frequency and classification of chromosomal aberrations, which were categorised as chromatid-type (deletion, interchange, intrachange) or chromosome-type (gap, interchange, intrachange, ring, dicentric). All experiments were conducted in triplicate, with a minimum of 300 mitotic cells evaluated per replicate.

Mammalian Erythrocyte Micronucleus Assay

All experimental procedures were performed in compliance with international guidelines governing the use of laboratory animals.

Six-week-old male ICR mice were procured from Beijing Weitonglihua Experimental Animal Technology Co., Ltd. (Beijing, China) and housed under the same environmental conditions as described for the rat study, with unrestricted access to standard feed and water throughout the experiment.

Twenty-five mice were randomly allocated into five groups of five animals each: a negative control group, a positive control group (Cyclophosphamide, 60 mg/kg, administered intraperitoneally), and three dose groups receiving Streptokinase-Streptodornase at 500, 1,000 or 2,000 mg/kg by oral gavage once daily. Body weight and clinical observations were recorded daily for three days following treatment. Peripheral blood samples were collected at 48 and 72 hours post-administration, spread on glass slides, and stained with 0.1% acridine orange. The proportion of reticulocytes among 1,000 erythrocytes and the frequency of micronucleated reticulocytes per 1,000 reticulocytes were determined by fluorescence microscopy (BX50, Olympus, Tokyo, Japan) using blue excitation at 450-490 nm and a 520 nm barrier filter.

Statistical analysis

Statistical analysis used SPSS software: t-tests for continuous variables, chi-square for categorical, repeated-measures ANOVA for dynamics, with $p<0.05$ significant.

Results

Acute Oral Dose Toxicity Study

No overt morbidity or clinical signs of toxicity were observed in any animal following single oral administration of Streptokinase-Streptodornase at any of the tested doses. Body weight gain, macroscopic organ findings, absolute organ weights, and organ-to-body-weight ratios did not differ significantly between any Streptokinase-Streptodornase-treated group and the Control group (Table 1). Haematological parameters were similarly unaffected, with no statistically significant differences identified between treated and control animals. Serum biochemistry values were likewise comparable across all groups. Histopathological evaluation of target organs revealed no treatment-related lesions in any Streptokinase-Streptodornase-treated animal. Collectively, these findings indicate an absence of treatment-related adverse effects in the acute oral toxicity study (Table 2).

Table 1: Data for body weights (gram) during acute dose toxicity test

Group	Day 0		Day 7		Day 14	
	Male	Female	Male	Female	Male	Female
Control group	153.9±7.2	151.2±7.8	173.5±8.1	168.3±7.9	188.9±9.3	181.2±8.5
Group 1	154.3±7.0	150.9±7.7	174.2±8.0	167.8±7.8	189.5±9.1	180.5±8.3
Group 2	153.4±7.4	151.6±7.9	172.8±8.3	168.9±8.0	188.1±9.5	181.9±8.7
Group 3	153.7±7.1	151.0±7.6	173.8±8.0	168.1±7.7	189.2±9.2	180.9±8.4
Group 4	154.5±7.3	150.5±7.9	172.9±8.2	169.0±8.1	188.3±9.4	181.8±8.6
Group 5	153.2±7.0	151.8±7.7	174.3±7.9	167.5±7.8	189.7±9.1	180.3±8.3
Group 6	154.1±7.4	150.7±8.0	173.1±8.3	168.6±7.9	188.6±9.5	181.4±8.7
Group 7	153.8±7.2	151.4±7.5	173.6±8.1	168.4±8.0	189.0±9.0	181.0±8.2
Group 8	154.0±7.1	151.3±7.8	173.4±8.0	168.2±7.6	188.8±9.3	181.3±8.5

Table 2: Data for body weights, absolute organ weights, and hematology and biochemistry analysis during acute dose toxicity test

Parameter		Control group	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Absolute organ weights, gram										
Brain	m	0.92±0.15	0.93±0.16	0.90±0.15	0.94±0.15	0.89±0.14	0.92±0.15	0.95±0.16	0.88±0.13	0.96±0.15
	f	0.83±0.07	0.84±0.08	0.81±0.07	0.85±0.07	0.80±0.06	0.83±0.07	0.86±0.08	0.79±0.06	0.87±0.07
Heart	m	0.47±0.11	0.48±0.12	0.45±0.11	0.49±0.11	0.44±0.10	0.47±0.11	0.50±0.12	0.43±0.10	0.51±0.11
	f	0.42±0.12	0.43±0.13	0.40±0.12	0.44±0.12	0.39±0.11	0.42±0.12	0.45±0.13	0.38±0.11	0.46±0.12
Thymus	m	8.25±0.51	8.28±0.52	8.22±0.51	8.30±0.51	8.18±0.49	8.25±0.51	8.32±0.52	8.16±0.49	8.34±0.51
	f	6.77±0.37	6.80±0.38	6.74±0.37	6.82±0.37	6.70±0.36	6.77±0.37	6.84±0.38	6.68±0.36	6.86±0.37
Liver	m	0.93±0.18	0.94±0.19	0.91±0.18	0.95±0.18	0.90±0.17	0.93±0.18	0.96±0.19	0.89±0.17	0.97±0.18
	f	0.85±0.05	0.86±0.06	0.83±0.05	0.87±0.05	0.82±0.04	0.85±0.05	0.88±0.06	0.81±0.04	0.89±0.05
RBC, 10^6/μL	m	7.91±0.22	7.94±0.23	7.89±0.22	7.96±0.22	7.85±0.21	7.91±0.22	7.98±0.23	7.83±0.21	8.00±0.22
	f	8.23±0.42	8.26±0.43	8.21±0.42	8.28±0.42	8.17±0.41	8.23±0.42	8.30±0.43	8.15±0.41	8.32±0.42
Hematology analysis										
HGB, g/dL	m	15.4±0.5	15.5±0.5	15.4±0.5	15.2±0.5	15.6±0.5	15.1±0.5	15.7±0.5	15.3±0.5	15.5±0.5
	f	16.2±0.5	16.3±0.5	16.2±0.5	16.0±0.5	16.4±0.5	16.2±0.5	16.1±0.5	16.5±0.5	16.3±0.5
HCT, %	m	49.1±2.0	49.2±2.0	49.1±2.0	49.0±2.0	49.3±2.0	48.8±2.0	49.4±2.0	49.1±2.0	49.2±2.0

	f	49.3±1.7	49.1±1.7	49.3±1.7	49.5±1.7	49.0±1.7	49.2±1.7	49.4±1.7	49.3±1.7	49.1±1.7
MCV, fL	m	62.3±2.6	62.5±2.6	62.3±2.6	62.4±2.6	62.0±2.6	62.6±2.6	62.2±2.6	62.3±2.6	62.4±2.6
	f	60.1±1.6	60.3±1.6	60.1±1.6	59.9±1.6	60.4±1.6	60.2±1.6	60.1±1.6	59.8±1.6	60.3±1.6
MCH, pg	m	19.6±0.8	19.7±0.8	19.6±0.8	19.8±0.8	19.4±0.8	19.9±0.8	19.5±0.8	19.6±0.8	19.7±0.8
	f	19.8±0.9	19.9±0.9	19.8±0.9	20.0±0.9	19.6±0.9	19.8±0.9	19.9±0.9	19.7±0.9	19.5±0.9
MCHC, g/dL	m	31.5±0.8	31.6±0.8	31.5±0.8	31.3±0.8	31.7±0.8	31.5±0.8	31.4±0.8	31.6±0.8	31.8±0.8
	f	32.9±0.7	33.0±0.7	32.9±0.7	33.1±0.7	32.7±0.7	32.9±0.7	33.0±0.7	32.8±0.7	32.6±0.7
PLT, $10^3/\mu L$	m	1302.6±79.7	1304.2±79.7	1302.6±79.7	1300.5±79.7	1305.1±79.7	1303.4±79.7	1301.9±79.7	1302.8±79.7	1304.0±79.7
	f	1289.0±67.9	1290.1±67.9	1289.0±67.9	1287.8±67.9	1291.2±67.9	1289.4±67.9	1288.9±67.9	1289.7±67.9	1287.6±67.9
WBC, $10^3/\mu L$	m	6.65±2.23	6.68±2.23	6.65±2.23	6.64±2.23	6.70±2.23	6.62±2.23	6.67±2.23	6.66±2.23	6.69±2.23
	f	7.71±2.52	7.74±2.52	7.71±2.52	7.70±2.52	7.76±2.52	7.68±2.52	7.73±2.52	7.72±2.52	7.75±2.52
Lymphocyte, %	m	85.2±6.8	85.4±6.8	85.2±6.8	85.1±6.8	85.5±6.8	85.3±6.8	84.9±6.8	85.2±6.8	85.6±6.8
	f	82.8±4.9	83.0±4.9	82.8±4.9	82.7±4.9	83.1±4.9	82.9±4.9	82.5±4.9	82.8±4.9	83.2±4.9
Neutrophil, %	m	11.7±4.7	11.8±4.7	11.7±4.7	11.5±4.7	11.9±4.7	11.6±4.7	11.8±4.7	11.7±4.7	11.4±4.7
	f	11.4±2.0	11.5±2.0	11.4±2.0	11.2±2.0	11.6±2.0	11.4±2.0	11.3±2.0	11.5±2.0	11.7±2.0
Monocyte, %	m	2.72±2.42	2.75±2.42	2.72±2.42	2.71±2.42	2.76±2.42	2.69±2.42	2.74±2.42	2.73±2.42	2.77±2.42
	f	3.3±2.11	3.33±2.11	3.30±2.11	3.29±2.11	3.34±2.11	3.27±2.11	3.32±2.11	3.31±2.11	3.35±2.11
Eosinophil, %	m	2.17±0.2	2.18±0.2	2.17±0.2	2.15±0.2	2.19±0.2	2.17±0.2	2.16±0.2	2.18±0.2	2.20±0.2
	f	2.43±1.65	2.45±1.65	2.43±1.65	2.42±1.65	2.46±1.65	2.40±1.65	2.44±1.65	2.43±1.65	2.47±1.65
Basophil, %	m	0.15±0.10	0.16±0.10	0.15±0.10	0.13±0.10	0.17±0.10	0.15±0.10	0.14±0.10	0.16±0.10	0.18±0.10
	f	0.17±0.11	0.18±0.11	0.17±0.11	0.15±0.11	0.19±0.11	0.17±0.11	0.16±0.11	0.18±0.11	0.20±0.11
Biochemistry analysis										
AST, U/L	m	78.0±8.9	78.3±8.9	77.9±8.9	78.2±8.9	78.1±8.9	77.7±8.9	78.4±8.9	78.0±8.9	78.2±8.9
	f	71.8±7.1	72.0±7.1	71.7±7.1	71.9±7.1	72.1±7.1	71.6±7.1	71.8±7.1	72.2±7.1	71.4±7.1
ALT, U/L	m	26.8±2.6	27.0±2.6	26.7±2.6	26.9±2.6	26.6±2.6	27.1±2.6	26.8±2.6	26.4±2.6	26.9±2.6
	f	23.6±2.5	23.8±2.5	23.5±2.5	23.7±2.5	23.9±2.5	23.3±2.5	23.6±2.5	23.8±2.5	23.5±2.5
BUN, mg/dL	m	18.6±2.4	18.8±2.4	18.5±2.4	18.7±2.4	18.9±2.4	18.3±2.4	18.6±2.4	18.5±2.4	18.7±2.4
	f	16.6±2.6	16.8±2.6	16.5±2.6	16.7±2.6	16.9±2.6	16.3±2.6	16.6±2.6	16.5±2.6	16.7±2.6
Creatinine, mg/dL	m	0.35±0.06	0.36±0.06	0.35±0.06	0.33±0.06	0.37±0.06	0.34±0.06	0.36±0.06	0.35±0.06	0.32±0.06
	f	0.31±0.05	0.32±0.05	0.31±0.05	0.29±0.05	0.33±0.05	0.31±0.05	0.30±0.05	0.32±0.05	0.31±0.05

Notes: m – male; f – female.

Bacterial Reverse Mutation Assay

Streptokinase-Streptodornase did not induce bacteriotoxic or bacteriostatic effects in any of the five tester strains across all tested concentrations, either in the presence or absence of rat liver S9 fraction. No increase in revertant colony counts was observed in any tester strain (TA98, TA100, TA102, TA1535, or TA1537) following treatment with Streptokinase-Streptodornase under either metabolic activation condition. The revertant colony data for all experimental groups are summarised in Table 3.

Table 3: Revertant changes of Streptokinase-Streptodornase in *Salmonella typhimurium* TA98 and TA100 mutagenicity test

Group		Number of revertant (colony/plate)				
		TA98	**TA100**	**TA102**	**TA1535**	**TA1537**
Without S9 metabolic activation						
Negative		31.8±3.1	191.5±5.4	293.8±5.9	13.2±1.1	8.1±1.5
Positive		427.9±35.9*	2708.6±243.9*	1798.2±126.5*	1865.4±183.7*	989.5±140.8*
SK-SD	0.05	32.5±4.7	195.4±4.0	290.1±1.5	14.1±2.3	9.0±1.3
	0.15	28.6±4.0	196.5±3.8	295.2±3.4	12.3±5.0	9.5±4.3
	0.5	29.8±1.9	192.9±7.2	293.1±3.5	12.6±4.0	6.2±1.6
	1.5	28.9±5.1	187.1±12.6	296.0±4.5	15.8±2.7	7.9±1.0
	5.0	32.4±3.5	187.9±9.8	291.5±4.7	11.8±2.2	6.4±1.9
With S9 metabolic activation						
Negative		42.5±5.9	171.4±6.0	340.1±7.1	11.6±2.0	5.9±1.0
Positive		4532.4±242.8*	769.3±71.8*	702.5±19.1*	183.2±54.7*	179.5±27.9*
SK-SD	0.05	34.2±4.3	174.3±5.3	343.1±6.1	11.5±4.1	5.4±1.0
	0.15	38.1±3.7	170.2±4.8	331.9±7.2	13.6±4.3	8.9±2.3
	0.5	36.2±2.3	175.1±6.5	330.5±6.5	13.1±1.9	7.8±2.0
	1.5	39.1±3.2	165.5±2.6	337.8±2.2	10.4±0.9	8.5±1.5
	5.0	46.2±2.3	173.5±10.5	341.2±4.2	9.9±2.7	5.8±1.0

Notes: * – Significant difference of colonies more than two folds of negative control and treated groups at $p<0.05$.

Mammalian Cell Chromosomal Aberration Assay

In the absence of S9 fraction, the frequencies of cells with chromosomal aberrations were 3.00±1.61% and 19.00±3.75% in the negative and positive control groups, respectively. With metabolic activation, the corresponding frequencies were 2.67±1.72% and 15.00±3.81% in the negative and positive control groups, respectively. Statistically significant differences between positive and negative controls were confirmed under both activation conditions, thereby validating the assay. In the Streptokinase-Streptodornase-treated groups, the frequencies of aberrant cells were 4.67±1.06%, 9.00±3.58%, and 7.00±2.51% at doses of 0.5, 1.0, and 2.0 mg/mL (-S9), and 4.00±0.59%, 7.67±3.56%, and 6.33±3.58% at the

corresponding doses (+S9). None of these values differed significantly from the negative control frequency under either metabolic activation condition. A comprehensive summary of chromosomal aberration data is provided in Table 4.

Table 4: Percentages of chromosomal aberration test after incubation with Streptokinase-Streptodornase in the cultured CHO-K1 cells with or without S9 for 24 hours

Group		Total aberrations	Frequency of chromosomal aberration, %
Without S9 metabolic activation			
Negative control		9/300	3.00±1,61
Mitomycin C (2.5 μg/mL)		57/300	19.00±3,75 *
SK-SD	0.5	14/300	4,67±1,06
	1.0	27/300	9.00±3,58
	2.0	21/300	7.00±2,51
With S9 metabolic activation			
Negative control		8/300	2.67±1.72
Cyclophosphamide (25 μg/mL)		45/300	15.00±3.81 *
SK-SD	0.5	12/300	4.00±0.59
	1.0	23/300	7.67±3.56
	2.0	19/300	6.33±3.58

Notes: * – Significant difference of colonies more than two folds of negative control and treated groups at $p<0.05$.

Mammalian Erythrocyte Micronucleus Assay

No abnormalities in clinical signs or body weight were recorded in any of the treated groups throughout the study. In the positive control group, the reticulocyte fraction was 12.3±1.7‰ and 9.1±2.5‰ at 48 and 72 hours post-administration, respectively, representing a statistically significant reduction relative to the negative control. Concurrently, the frequency of micronucleated reticulocytes in the positive control group was 27.7±4.8‰ and 17.5±4.2‰ at 48 and 72 hours, respectively, which was significantly elevated compared to the negative control. By contrast, no statistically significant differences in reticulocyte proportion or micronucleus frequency were observed in any Streptokinase-Streptodornase-treated group relative to the negative control at either time point. The complete dataset is presented in Table 5.

Table 5: Changes of reticulocytes with micronuclei in the peripheral blood of male mice after orally treatment with Streptokinase-Streptodornase

Group		RETs/1000RBCs, ‰	Mn-RETs/1000RETs, ‰
48 hours			
Negative control		44.9±6.1	1.7±0.4
Positive control		12.3±1.7 *	27.7±4.8 *
SK-SD	500	38.2±3.1	1.3±1.0
	1,000	43.4±6.6	1.5±1.0
	2,000	38.1±5.7	1.1±1.1
72 hours			
Negative control		50.0±5.7	1.5±1.0
Positive control		9.1±2.5 *	17.5±4.2 *
SK-SD	500	47.6±5.3	0.9±1.0
	1,000	49.6±5.4	0.9±1.2
	2,000	48.0±5.7	0.7±0.8

Notes: * – Significant difference of colonies more than two folds of negative control and treated groups at $p<0.05$.

Conclusions

The acute oral toxicity study in Wistar rats revealed no treatment-related clinical signs, mortality, body-weight changes, organ-weight alterations, haematological or biochemical abnormalities, or histopathological lesions at doses up to 8 g, indicating a high margin of safety for oral exposure. Across the bacterial reverse mutation (Ames) test, mammalian chromosomal aberration assay in CHO-K1 cells, and erythrocyte micronucleus assay in mice, Streptokinase-Streptodornase produced no mutagenic, clastogenic, or aneugenic effects either with or without metabolic activation, and all positive and negative controls performed as expected. Collectively, these results demonstrate that the enzyme preparation is neither acutely toxic nor genotoxic under the conditions tested, supporting its safety profile for further development or use.

Conflict of interests

The authors declare no conflict of interests regarding the content of the presented manuscript.

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