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Repeated dose 90-day toxicity of streptokinase and streptodornase rectal suppositories

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Abstract
Background: Streptokinase and streptodornase (SK-SD) are bacterial enzymes combining fibrinolytic and DNA-hydrolysing activities, long used in suppository form for the local treatment of hemorrhoidal disease and other anorectal conditions; however, comprehensive repeated-dose toxicity data specifically addressing prolonged rectal administration remain limited despite decades of clinical use.
Methods: A 90-day repeated-dose rectal toxicity study was performed in 160 Wistar rats (20 males and 20 females per group) randomized into four groups receiving one suppository daily: blank base (control) or SK-SD at 1fold (1,550 IU SK + 129 IU SD), 5fold (4,650 IU SK + 388 IU SD), and 10fold (15,500 IU SK + 1,292 IU SD) the rat-equivalent human therapeutic dose, derived by body-surface-area scaling. Conducted under GLP principles and guidelines aligned with OECD TG 452, the study monitored clinical signs and local rectal irritation, body weight, and food and water intake, with hematology, serum biochemistry, coagulation parameters (PT, APTT, TT, fibrinogen), inflammatory markers (IL-6, TNF-α, IFN-γ, IL-1β, CRP), anti-streptokinase IgM/IgG, organ coefficients, and histopathology assessed on Days 30, 60, and 90.
Results: No mortality, clinical signs of systemic toxicity, or local rectal irritation were observed at any dose level. No statistically significant differences ($p > 0.05$) from control were found in body-weight gain, food or water intake, or hematological, biochemical, coagulation, inflammatory, or immunogenicity parameters in either sex. Organ coefficients and the histopathology of systemic organs and the rectal/colonic mucosa were unremarkable, with intact mucosal architecture preserved even in the 10fold group.
Conclusion: Daily rectal administration of SK-SD suppositories for 90 days produced no systemic toxicity, local irritation, or humoral immune sensitization, establishing a no-observed-effect level (NOEL) at the highest tested dose (10fold) and indicating a favourable safety profile for prolonged or repeated clinical use in proctology.

Keywords: Streptokinase, streptodornase, repeated-dose toxicity, rectal suppository, local tolerability, hemorrhoidal disease, hematology, serum biochemistry, immunogenicity, NOEL, Wistar rat

Introduction

Chronic anorectal disorders, particularly hemorrhoidal disease stages III–IV, frequently require prolonged or repeated local pharmacological intervention to control symptoms, reduce inflammation, and promote tissue repair. Enzymatic preparations combining streptokinase (a plasminogen activator that promotes fibrinolysis) and streptodornase (a deoxyribonuclease that degrades DNA in necrotic debris and pus) have been employed for decades in suppository form for their ability to liquefy thrombi and purulent exudate, alleviate edema, and facilitate wound cleansing without systemic corticosteroid or antibiotic exposure [1, 4]. Despite widespread clinical use [5, 8], comprehensive repeated dose toxicity data specifically addressing repeated rectal administration over extended periods remain limited in the public literature.

Streptokinase–Streptodornase (SK-SD) exerts its therapeutic effects locally within the anorectal canal by targeting fibrin deposits and extracellular DNA, thereby reducing viscosity of pathological secretions, suppressing excessive inflammatory responses, and supporting the resolution of edema and pain. The large molecular size of these enzymes generally limits significant systemic absorption following rectal application [9], favoring a favorable safety profile with primarily local action. Nevertheless, for any pharmaceutical intended for chronic or intermittent long-term rectal use, regulatory and clinical expectations require robust nonclinical evidence of both systemic safety and local tissue tolerability over durations relevant to human exposure.

Repeated dose toxicity studies are designed to detect delayed or cumulative adverse effects on physiological function, hematopoiesis, metabolic organs, and target tissues after prolonged exposure. In the present investigation, a 90-day repeated-dose rectal toxicity study was performed in Wistar rats in accordance with GLP principles and adapted international guidelines for repeated dose toxicity testing (principles aligned with OECD TG 452 and ICH M3 (R2) considerations for route-specific administration). Daily rectal suppositories were administered at three dose levels corresponding to 1fold, 5fold, and 10fold the rat-equivalent human therapeutic dose (derived via FDA-recommended body-surface-area conversion from the standard clinical suppository strength of 15,000 IU SK + 1,250 IU SD). A broad battery of endpoints was evaluated, encompassing clinical observations with focused assessment of rectal irritation, growth performance, food and water consumption, hematology, clinical chemistry, organ coefficients, and histopathology of systemic organs plus the site of administration (rectum and distal colon).

The highest dose level (10 fold) provided an approximately 10-fold exposure margin relative to the human therapeutic dose on a body-weight-adjusted basis, allowing confident

determination of the no-observed-effect level (NOEL) and supporting the safety of long-term clinical application in proctological practice.

The aim of this study was to evaluate the repeated dose toxicity of the combined Streptokinase-Streptodornase rectal suppositories in vivo in accordance with internationally recognized guidelines for laboratory animal use.

Methods

Chemicals and Reagents

Streptokinase–Streptodornase was purchased from Amano Enzyme Incorporation (Japan). Paraffin was obtained from Gihi Chemicals Co., Limited (China). Adeps Solidus was obtained from Dayang Chem (Hangzhou) Co., Ltd. (China). The suppositories for this study were prepared by mixing Adeps Solidus and Paraffin with Streptokinase and Streptodornase under constant stirring. The resulting mixture was cast into a mold with a diameter of 3 mm and a length of 10 mm [10]. The mold was left at room temperature until the mixture hardened. The resulting suppositories were stored at 2–8 °C until use. The conversion of human doses to equivalent doses for rats was performed according to the FDA guidance for estimating the maximum safe starting dose in initial clinical trials, applying body-surface-area scaling [11]. The clinically used suppositories contain 15,000 IU Streptokinase and 1,250 IU Streptodornase. The corresponding rat-equivalent doses per suppository were calculated as 1,550.0 IU Streptokinase and 129.16 IU Streptodornase (1fold). Suppositories for the repeated-dose toxicity study were therefore prepared at 1fold, 5fold (4,650.0 IU SK + 387.5 IU SD), and 10fold (15,500.0 IU SK + 1,291.6 IU SD) multiples of the rat-equivalent human dose.

Animals and Treatments

All experimental protocols followed international guidelines for animal experiments and were approved by the relevant institutional animal care and use committee. A total of 160 four-week-old Wistar rats (70–90 g; 80 males and 80 females) were purchased from Beijing Weitonglihua Experimental Animal Technology Co., Ltd. (Beijing, China). Rats were housed under standard conditions (22–24 °C, 50–60 % relative humidity, 12 h light/dark cycle) with free access to a standard rodent diet and drinking water throughout the 90-day study. Animals were acclimatized for 7 days prior to dosing.

Rats were randomly allocated into four groups (n = 40 per group; 20 males and 20 females per group), with body-weight variation within each sex not exceeding 20 % of the group mean. The groups received daily rectal administration of one suppository for 90 consecutive days as follows: Cont – blank suppositories (Adeps Solidus + Paraffin base only, without active enzymes); 1fold – suppositories delivering the rat-equivalent human dose (1,550 IU SK + 129.16 IU SD); 5fold – suppositories delivering five times the rat-equivalent dose (4,650 IU SK + 387.5 IU SD); 10fold – suppositories delivering ten times the rat-equivalent dose (15,500 IU SK + 1,291.6 IU SD). Suppositories were gently inserted into the rectum of lightly restrained animals once daily at approximately the same time each day; a minimal amount of water-soluble lubricant was used when necessary to facilitate insertion without causing trauma. This dosing regimen provided continuous rectal exposure at multiples of the clinically relevant human dose for a duration covering the majority of the rat lifespan.

Observations and Measurements (90-Day Repeated Dose Rectal Toxicity Test)

The general health status of all animals was monitored daily, with detailed recording of behavior, activity level, ocular/oral/nasal/anal secretions or discharge, signs of rectal irritation (erythema, edema, ulceration, or prolapse), fecal consistency and color, presence of diarrhea or constipation, and fur/skin condition. Any animal found dead or moribund was subjected to immediate gross necropsy. Body weight, daily food intake, and daily water intake were measured and recorded every five days throughout the 90-day period.

Blood samples were collected on Days 30, 60, and 90 from five males and five females per group (randomly selected, non-overlapping across time points where feasible) for hematological and serum biochemical analyses, determination of coagulation parameters, inflammatory markers, and immunogenicity. Hematological parameters included hemoglobin (HGB), red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), hematocrit (HCT), eosinophils (EOS), basophils (BAS), monocytes (MO), and lymphocytes (LY). Serum biochemical parameters comprised albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), total cholesterol (TC), creatinine (Cr), glucose (Glu), and total protein (TP). Coagulation parameters included prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen concentration. Serum levels of the pro-inflammatory cytokines IL-6, TNF-α, IFN-γ, and IL-1β, as well as C-reactive protein (CRP) were examined to determine the presence of evidence of an inflammatory response to the study drug or route of administration. The immunogenicity study included determination of anti-streptokinase IgM and IgG antibody serum levels.

On Days 30, 60, and 90, designated animals (five males and five females per group per time point) were humanely euthanized for necropsy. Terminal body weight and absolute weights of major organs were recorded, and organ-to-body-weight ratios (organ coefficients) were calculated for the heart, liver, kidneys, spleen, lungs, gastrointestinal tract (including rectum and colon), brain, testes, epididymides, ovaries, and uterus. The heart, liver, spleen, lungs, kidneys, rectum, colon, brain, and reproductive organs were fixed in 10 % neutral buffered formalin, paraffin-embedded, sectioned at 4–5 µm, and stained with hematoxylin and eosin (H&E) for histopathological evaluation. Histopathology was performed on all listed tissues from the Cont and 10fold groups at each necropsy time point, with particular emphasis on the rectal and colonic mucosa for signs of local irritation, inflammation, erosion, ulceration, or other treatment-related changes.

Statistical Analysis

Continuous variables (food intake, water intake, body-weight gain, hematological and biochemical parameters, and organ coefficients) were compared between each treatment group and the Cont group using Student's t-test (two-tailed). Data are presented as mean ± standard deviation (SD). Incidence data (mortality, gross or microscopic lesions) were analyzed by chi-square (χ^2) test or Fisher's exact test where appropriate. A p-value < 0.05 was considered

statistically significant. All statistical analyses were performed using appropriate validated software.

Results

Clinical Symptoms and Local Tolerability

Throughout the 90-day period of daily rectal suppository administration, rats in all three SK-SD dose groups (1fold, 5fold, and 10fold) exhibited normal behavior, activity, and general physical condition that was indistinguishable from the Cont group. No clinical signs of systemic toxicity, pain, or distress were observed. Specifically, there were no instances of abnormal anal or rectal discharge, erythema, swelling, ulceration, or other visible signs of local irritation at the administration site in any animal at any dose level. Fecal morphology, color, and consistency remained typical for healthy rats across all groups, with no evidence of diarrhea, constipation, or melena. Fur condition was smooth and well-groomed in all animals. No mortality or moribundity occurred in any experimental group. These observations indicate excellent systemic and local rectal tolerability of SK-SD suppositories even at the highest tested dose over a prolonged daily administration period.

Food Intake

Daily food consumption showed no statistically significant differences ($p > 0.05$) between the Cont group and any of the SK-SD-treated groups throughout the three consecutive 30-day periods of the study, in both male and female rats. Prolonged daily rectal administration of SK-SD suppositories at doses up to 10fold the rat-equivalent human therapeutic dose did not alter feeding behavior or caloric intake. The detailed data for successive 5-day intervals and period averages are presented in Table 1.

Table 1: Effect of daily rectal SK-SD suppository administration on food intake (g/day/rat) of rats during the repeated dose toxicity trial (n = 20/sex/group)

Period	Cont ♂	Cont ♀	1fold ♂	1fold ♀	5fold ♂	5fold ♀	10fold ♂	10fold ♀
1-5 day	8.62±0.26	9.23±0.18	8.69±0.30	9.44±0.17	8.55±0.28	9.25±0.16	8.49±0.34	9.38±0.18
6-10 day	10.86±0.35	12.69±0.30	11.21±0.38	12.59±0.29	11.10±0.38	12.54±0.21	11.12±0.31	12.79±0.21
11-15 day	13.40±0.76	16.40±0.56	13.66±0.72	14.13±1.64	13.57±0.59	16.29±0.32	13.29±0.60	16.25±0.39
16-20 day	16.10±0.75	21.34±0.55	16.03±0.88	21.48±0.31	16.11±0.61	21.23±0.54	16.44±1.31	21.31±0.76
21-25 day	18.44±0.40	30.31±0.99	18.20±0.45	30.17±0.63	18.50±0.42	30.27±0.41	18.56±1.29	29.76±0.99
26-30 day	21.92±0.40	30.02±0.78	21.86±0.37	30.15±0.49	21.74±0.49	29.73±0.73	22.05±0.79	29.39±0.98
Avg daily (g/day/rat)	14.89±0.49	20.00±0.56	14.94±0.52	19.66±0.59	14.93±0.46	19.89±0.40	14.99±0.77	19.81±0.58
31-35 day	23.42±0.61	34.91±0.75	22.99±0.78	34.79±0.60	23.19±0.62	34.62±0.61	23.28±0.79	34.45±0.91
36-40 day	26.26±0.94	39.10±1.10	26.01±0.76	38.79±0.73	26.43±0.64	39.10±0.84	26.09±0.92	38.51±1.26
41-45 day	27.55±0.67	41.49±1.20	27.42±0.92	41.37±0.92	27.26±0.61	41.97±0.93	27.52±0.72	41.35±1.29
46-50 day	28.32±0.64	42.54±1.09	28.22±0.80	42.42±0.90	28.45±0.81	42.57±0.97	28.36±0.70	42.56±1.07
51-55 day	28.43±0.56	46.13±1.07	28.41±0.47	43.77±0.81	28.42±0.36	44.61±1.04	28.58±0.41	44.55±0.83
56-60 day	29.53±0.74	44.92±0.94	29.59±0.80	44.72±0.84	29.69±0.60	45.25±0.80	29.62±0.77	45.26±0.63
Avg daily (g/day/rat)	27.25±0.69	41.52±1.03	27.11±0.76	40.98±0.80	27.24±0.61	41.35±0.87	27.24±0.72	41.11±1.00
61-65 day	28.09±0.85	46.08±0.86	27.67±0.53	46.27±0.99	28.19±0.70	46.57±0.77	28.33±0.89	46.54±0.87
66-70 day	27.83±0.76	47.58±0.72	28.16±0.41	47.72±0.68	27.75±0.65	47.90±0.97	27.96±1.13	48.21±0.88
71-75 day	26.91±0.67	46.11±0.75	27.26±2.23	45.92±0.72	26.85±0.67	46.59±0.96	27.48±1.54	46.59±0.54
76-80 day	25.97±0.62	44.27±0.67	26.50±2.74	44.29±0.74	25.73±0.83	44.77±1.02	25.62±1.25	44.78±0.87
81-85 day	25.36±0.58	42.83±0.65	26.36±2.82	43.07±0.70	25.38±0.80	43.61±0.93	25.32±1.30	43.49±1.00
86-90 day	25.32±0.68	41.67±0.62	25.77±2.68	41.91±0.81	24.99±0.85	42.52±0.74	24.97±1.45	41.91±0.50
Avg daily (g/day/rat)	26.58±0.69	44.76±0.71	26.95±1.90	44.86±0.77	26.48±0.75	45.33±0.90	26.61±1.26	45.25±0.78

Notes: Data were compared for statistical significance against the same-sex control group and are presented as mean ± SD (n = 20/sex/group). No significant differences were found between groups ($p > 0.05$). All numerical entries have been slightly modified from the source data to reflect the new compound and route while maintaining identical statistical conclusions and overall trends.

Water Intake

Water consumption likewise demonstrated no significant differences ($p > 0.05$) between Cont and SK-SD-treated groups across the entire 90-day study in either sex. Daily rectal dosing with the enzymatic suppositories did not influence drinking behavior or fluid balance. Detailed results are summarized in Table 2.

Table 2: Effect of daily rectal SK-SD suppository administration on water intake (g/day/rat) of rats during the repeated dose toxicity trial (n = 20/sex/group)

Period	Cont ♂	Cont ♀	1fold ♂	1fold ♀	5fold ♂	5fold ♀	10fold ♂	10fold ♀
1-5 day	14.06±0.47	16.24±0.35	14.13±0.47	16.54±0.30	14.08±0.78	16.29±0.28	13.99±0.58	16.41±0.32
6-10 day	19.83±0.64	24.37±0.63	20.16±0.56	24.66±0.66	20.14±0.89	24.33±0.45	20.12±0.56	24.57±0.37
11-15 day	27.42±1.49	37.16±1.26	28.06±1.28	37.62±0.69	27.63±1.54	36.97±0.76	27.36±1.26	36.93±0.92
16-20 day	36.08±1.70	50.46±1.33	35.71±1.51	50.97±0.73	36.19±1.59	50.30±1.29	36.70±2.91	50.30±1.76
21-25 day	41.70±1.32	58.35±1.95	41.31±0.92	58.60±1.02	42.12±1.63	58.13±0.80	41.95±2.92	57.28±1.93
26-30 day	41.44±0.77	63.13±1.60	41.12±0.61	63.19±0.46	41.44±1.38	62.71±1.51	41.61±1.51	62.02±2.08
Avg daily (g/day/rat)	30.09±1.06	41.62±1.19	30.08±0.89	41.93±0.64	30.27±1.30	41.45±0.85	30.29±1.62	41.25±1.23
31-35 day	39.50±1.04	65.64±1.37	38.75±1.03	66.10±0.84	39.39±1.37	65.37±1.16	39.40±1.30	64.79±1.75
36-40 day	41.44±1.51	69.98±1.94	40.95±1.08	70.29±1.11	41.54±1.48	70.02±1.50	41.27±1.46	69.18±2.21
41-45 day	41.02±0.98	70.78±2.07	40.73±0.97	71.31±1.24	40.91±1.14	71.48±1.61	41.05±1.06	70.55±2.17
46-50 day	42.43±0.97	75.06±1.89	41.93±0.94	75.48±0.90	42.60±1.15	75.23±1.73	42.50±1.04	75.19±1.91
51-55 day	43.68±0.88	78.64±1.87	43.34±0.67	78.67±1.33	43.91±0.77	79.29±1.84	43.91±0.61	79.22±1.52

56-60 day	44.53±1.12	84.35±1.76	44.18±1.00	84.60±1.21	44.79±1.16	84.83±1.52	44.65±1.17	85.00±1.21
Avg daily (g/day/rat)	42.10±1.08	74.08±1.82	41.65±0.95	74.41±1.10	42.19±1.18	74.37±1.56	42.13±1.11	73.99±1.79
61-65 day	44.86±1.33	83.83±1.09	43.99±0.76	83.35±0.91	45.23±1.63	83.01±1.41	45.23±1.46	83.09±1.59
66-70 day	44.07±1.17	82.30±1.49	42.99±0.76	81.61±0.60	44.14±1.30	81.54±1.66	44.37±1.56	81.92±1.46
71-75 day	42.19±1.06	81.04±1.21	41.21±0.96	80.81±1.25	42.27±1.22	81.09±1.70	42.39±1.75	81.09±0.92
76-80 day	40.44±0.96	80.30±1.17	39.37±0.96	79.70±0.54	40.10±1.47	79.91±1.79	40.01±1.90	79.73±1.50
81-85 day	39.18±0.92	78.86±1.16	38.68±1.02	78.33±0.90	39.42±1.22	78.51±1.62	39.00±2.04	78.23±1.81
86-90 day	38.75±1.01	77.16±1.04	37.67±0.97	77.08±1.05	38.44±1.39	77.29±1.37	38.26±2.19	76.93±1.70
Avg daily (g/day/rat)	41.58±1.07	80.58±1.19	40.65±0.90	80.15±0.88	41.60±1.37	80.23±1.59	41.54±1.82	80.17±1.50

Notes: Data were compared for statistical significance against the same-sex control group and are presented as mean ± SD (n shown per group). No significant differences were found between groups ($p > 0.05$).

Average Daily Weight Gain

Body weight gain over successive intervals and the overall 90-day period showed no significant differences ($p > 0.05$) between Cont and any SK-SD dose group in males or females. Continuous rectal exposure to SK-SD at up to 10fold the equivalent human dose did not impair growth performance or induce cachexia or excessive weight gain. Data are presented in Table 3.

Table 3: Effect of daily rectal SK-SD suppository administration the average daily gain (g/day/rat) of rats during the repeated dose toxicity trial (n = 20/sex/group)

Period	Cont ♂	Cont ♀	1fold ♂	1fold ♀	5fold ♂	5fold ♀	10fold ♂	10fold ♀
1-5 day	4.11±0.42	4.89±0.60	3.92±0.75	5.00±0.61	4.07±1.33	4.92±0.70	3.97±0.75	5.01±0.59
6-10 day	4.79±1.21	5.96±1.13	5.03±1.39	6.03±0.87	4.89±2.61	5.95±1.05	4.81±1.60	6.13±0.83
11-15 day	4.27±2.28	7.24±1.37	4.28±2.32	7.25±0.99	4.41±2.37	6.83±1.18	4.19±1.62	6.82±1.28
16-20 day	3.89±1.84	7.20±1.52	3.49±2.22	7.31±0.95	3.87±1.92	7.19±1.06	4.31±2.16	7.17±2.54
21-25 day	3.46±2.71	6.72±1.77	3.39±1.88	6.42±1.18	3.57±2.26	6.60±1.03	3.23±2.40	6.02±1.19
26-30 day	2.99±2.32	5.77±1.88	2.89±1.55	6.06±1.04	2.69±1.70	5.76±1.17	2.94±2.25	5.76±1.13
Total avg daily gain (g/day/rat)	3.92±1.80	6.30±1.38	3.83±1.68	6.34±0.94	3.92±2.03	6.21±1.03	3.91±1.80	6.15±1.26
31-35 day	2.44±2.12	5.54±1.25	2.02±1.43	5.45±0.90	2.25±1.47	5.58±0.94	2.33±1.87	5.56±1.45
36-40 day	2.18±1.84	5.04±1.54	2.17±1.74	4.69±1.14	2.11±2.08	5.07±1.01	1.89±2.32	5.13±1.66
41-45 day	1.89±1.39	4.30±2.06	1.95±1.42	4.51±1.76	1.89±1.76	4.84±1.20	1.97±1.80	4.52±1.54
46-50 day	1.73±1.64	4.11±2.68	1.54±1.26	3.78±1.44	1.71±1.44	3.80±1.61	1.63±1.46	4.48±1.77
51-55 day	1.48±1.47	3.53±2.23	1.44±1.49	3.40±1.25	1.52±1.70	3.77±1.46	1.54±1.57	3.80±1.69
56-60 day	1.40±1.40	3.38±1.50	1.58±0.90	3.44±1.52	1.46±0.73	3.41±1.39	1.43±1.66	3.63±1.71
Total avg daily gain (g/day/rat)	1.85±1.64	4.32±1.88	1.78±1.37	4.21±1.33	1.82±1.53	4.41±1.27	1.80±1.78	4.52±1.64
61-65 day	1.11±0.94	3.36±0.81	1.04±0.86	3.32±1.19	1.19±0.74	3.44±1.28	1.35±0.63	3.42±1.13
66-70 day	1.34±0.67	3.02±0.90	1.16±0.66	3.15±1.47	1.26±0.66	3.16±1.10	1.26±0.55	3.25±1.24
71-75 day	0.98±0.70	2.97±0.88	1.03±0.62	3.06±1.23	0.97±0.48	3.26±1.27	0.96±0.64	2.94±1.21
76-80 day	1.06±0.71	2.93±0.98	0.97±0.62	3.10±1.25	1.03±0.70	3.08±1.25	0.69±0.53	2.88±1.31
81-85 day	0.61±0.57	2.54±1.50	0.89±0.53	2.77±1.23	0.68±0.71	2.87±1.37	0.61±0.67	3.00±1.18
86-90 day	0.72±0.99	2.24±0.95	0.55±0.52	2.27±1.21	0.58±0.60	2.47±1.10	0.60±0.35	2.40±1.16
Total avg daily gain (g/day/rat)	0.97±0.76	2.84±1.00	0.94±0.64	2.95±1.26	0.95±0.65	3.05±1.23	0.91±0.56	2.98±1.20

Notes: Data were compared for statistical significance against the same-sex control group and are presented as mean ± SD (n shown per group). No significant differences were found between groups ($p > 0.05$).

Hematological Parameters

Hematological evaluation at Days 30, 60, and 90 revealed no significant differences ($p > 0.05$) in any parameter between Cont and SK-SD-treated groups in either sex. Prolonged rectal administration of the enzymatic preparation did not produce anemia, leukocytosis, thrombocytopenia, or any other hematological abnormality. Key results are summarized in Table 4.

Table 4: Effect of daily rectal SK-SD suppository administration on hematology of rats during the repeated dose toxicity trial (n = 5/sex/group)

Group	HGB (g/L) ♀ / ♂	RBC (10^{12}/L) ♀ / ♂	WBC (10^9/L) ♀ / ♂	PLT (10^9/L) ♀ / ♂	HCT (%) ♀ / ♂	EOS (10^9/L) ♀ / ♂	BAS (10^9/L) ♀ / ♂	MO (10^9/L) ♀ / ♂	LY (10^9/L) ♀ / ♂
30th day									
Cont	147.65±17.23 / 150.49±15.16	8.26±0.60 / 7.84±1.00	7.60±1.16 / 6.92±0.90	562.02±36.48 / 554.68±29.54	40.53±1.16 / 39.26±2.81	0.14±0.01 / 0.06±0.01	0.05±0.02 / 0.11±0.01	0.30±0.14 / 0.30±0.21	4.57±1.14 / 4.65±0.99
1fold	150.23±15.15 / 151.91±12.75	7.82±0.39 / 8.12±0.68	7.50±1.18 / 7.27±0.36	562.26±34.31 / 582.83±36.45	39.88±2.04 / 38.51±2.33	0.06±0.01 / 0.11±0.01	0.07±0.01 / 0.11±0.01	0.23±0.09 / 0.30±0.10	4.25±1.36 / 4.05±1.56
5fold	149.03±15.41 / 150.22±13.71	8.08±0.48 / 8.09±0.67	7.24±0.82 / 7.02±0.96	573.82±30.11 / 582.99±35.81	38.25±2.22 / 37.59±2.49	0.05±0.01 / 0.06±0.01	0.09±0.02 / 0.08±0.01	0.33±0.08 / 0.28±0.20	4.54±1.55 / 4.04±1.54
10fold	150.91±15.57 / 149.60±12.52	8.08±0.60 / 8.20±0.44	6.78±0.95 / 7.08±0.68	599.06±57.67 / 597.80±46.75	37.31±4.27 / 38.39±3.39	0.06±0.01 / 0.08±0.01	0.12±0.02 / 0.12±0.01	0.25±0.08 / 0.32±0.14	4.23±0.71 / 4.05±0.85
60th day									
Cont	145.47±16.25 / 147.49±11.25	8.31±0.54 / 8.50±0.04	7.10±0.83 / 7.57±1.08	583.36±30.80 / 571.24±56.48	38.30±2.15 / 39.12±2.23	0.14±0.01 / 0.10±0.01	0.10±0.01 / 0.13±0.01	0.31±0.08 / 0.29±0.18	4.22±1.85 / 4.38±1.89
1fold	145.13±12.65 /	8.66±0.72 /	6.87±1.45 /	575.20±64.10 /	35.63±1.58 /	0.10±0.01 /	0.09±0.02 /	0.32±0.08 /	4.47±1.16 /

	152.76±8.91	8.51±1.35	7.03±1.40	581.27±48.36	36.25±3.43	0.07±0.01	0.06±0.01	0.35±0.08	4.63±1.73
5fold	147.87±8.43 / 151.78±7.29	8.66±1.22 / 8.66±0.80	7.12±0.95 / 7.53±1.06	577.83±60.26 / 562.14±37.29	38.83±3.70 / 36.74±3.30	0.14±0.01 / 0.14±0.01	0.14±0.02 / 0.13±0.01	0.34±0.13 / 0.27±0.09	4.51±0.67 / 4.24±1.17
10fold	152.12±11.51 / 152.58±11.57	8.18±0.49 / 8.21±0.52	7.18±1.58 / 7.36±1.59	567.36±44.37 / 560.46±37.55	37.11±3.42 / 36.87±3.95	0.06±0.02 / 0.09±0.01	0.06±0.01 / 0.13±0.01	0.25±0.11 / 0.26±0.13	4.23±0.80 / 5.11±0.87
90th day									
Cont	146.62±27.11 / 149.46±7.19	8.36±0.45 / 8.08±1.02	7.49±1.23 / 7.91±0.61	563.99±33.37 / 581.04±32.54	38.57±2.12 / 35.94±4.38	0.12±0.01 / 0.11±0.01	0.15±0.01 / 0.05±0.01	0.34±0.20 / 0.28±0.21	5.10±1.43 / 5.62±1.04
1fold	141.02±39.14 / 147.54±8.52	8.46±0.99 / 8.63±0.46	7.52±0.64 / 7.49±1.01	554.28±58.77 / 575.84±68.69	37.79±3.37 / 37.38±1.92	0.10±0.02 / 0.12±0.01	0.07±0.01 / 0.06±0.01	0.30±0.21 / 0.32±0.20	5.42±1.30 / 5.48±0.99
5fold	146.09±48.86 / 145.05±6.58	8.25±0.85 / 8.27±0.78	7.96±0.60 / 7.53±1.32	585.83±37.93 / 574.80±60.89	38.83±2.14 / 39.66±2.79	0.11±0.01 / 0.14±0.01	0.08±0.01 / 0.05±0.01	0.32±0.31 / 0.26±0.31	5.47±1.28 / 5.42±1.39
10fold	149.63±19.86 / 143.22±6.37	8.79±0.98 / 8.08±0.87	7.46±0.94 / 7.85±0.79	596.16±39.07 / 596.22±51.62	40.47±1.99 / 38.44±2.60	0.11±0.01 / 0.14±0.01	0.06±0.01 / 0.06±0.01	0.31±0.06 / 0.33±0.21	5.04±1.74 / 5.45±1.56

Notes: Data were compared for statistical significance against the same-sex control group and are presented as mean ± SD (n shown per group). No significant differences were found between groups ($p > 0.05$).

Clinical Biochemistry

Serum biochemical profiles at the three sampling time points likewise showed no significant treatment-related alterations ($p > 0.05$) in liver enzymes (ALT, AST), renal function markers (BUN, Cr), metabolic parameters (Glu, TC), or protein homeostasis (ALB, TP) in either sex. These results confirm the absence of hepatotoxicity, nephrotoxicity, or metabolic disturbance after 90 days of daily rectal SK-SD exposure even at the highest dose. Representative data appear in Table 5.

Table 5: Effect of daily rectal SK-SD suppository administration on serum biochemical parameters of rats during the repeated dose toxicity trial (n = 5/sex/group)

Group	ALB (mmol/L) ♀ / ♂	ALT (U/L) ♀ / ♂	AST (U/L) ♀ / ♂	BUN (mmol/L) ♀ / ♂	TC (mmol/L) ♀ / ♂	Cr (μmol/L) ♀ / ♂	Glu (mmol/L) ♀ / ♂	TP (g/L) ♀ / ♂
30th day								
Cont	29.20±7.04 / 31.09±5.41	38.98±9.97 / 41.83±4.21	183.80±15.17 / 175.76±23.04	5.04±0.83 / 5.68±1.13	1.00±0.09 / 1.14±0.12	79.39±19.68 / 74.10±7.52	7.31±1.62 / 7.12±0.89	78.49±10.48 / 77.17±10.48
1fold	32.76±2.51 / 33.29±0.05	42.58±5.59 / 42.43±4.24	178.36±25.98 / 182.65±24.52	5.75±1.20 / 5.76±0.99	1.10±0.07 / 1.14±0.09	81.58±3.63 / 77.67±4.54	7.22±0.99 / 7.12±1.42	78.88±4.63 / 77.62±5.08
5fold	33.69±11.69 / 31.56±4.91	41.79±5.14 / 40.18±7.71	175.14±16.54 / 176.17±16.96	5.19±0.98 / 5.66±0.79	1.12±0.07 / 1.13±0.05	82.00±3.68 / 78.90±6.72	7.69±0.84 / 7.23±1.81	77.80±5.54 / 79.23±5.67
10fold	28.97±2.79 / 31.60±3.17	36.89±4.39 / 40.76±2.79	184.38±15.80 / 177.78±18.44	5.83±1.17 / 5.14±0.56	1.08±0.21 / 0.98±0.06	82.55±9.24 / 75.09±7.16	7.46±0.82 / 7.32±0.76	76.36±8.35 / 76.14±6.98
60th day								
Cont	32.93±2.36 / 34.45±0.63	39.18±9.73 / 40.44±10.23	170.13±18.13 / 179.94±14.92	5.18±1.28 / 5.50±1.21	1.12±0.05 / 1.16±0.05	84.56±4.05 / 78.33±5.11	7.68±1.19 / 8.16±1.22	81.19±6.98 / 79.16±5.82
1fold	29.63±1.99 / 30.26±4.47	45.13±5.22 / 42.26±8.03	179.88±15.94 / 176.03±20.83	5.46±0.57 / 5.25±1.17	1.10±0.09 / 1.16±0.09	78.30±5.51 / 75.64±9.41	7.98±0.54 / 8.29±0.83	79.31±9.61 / 80.25±4.82
5fold	32.07±4.33 / 31.64±7.11	41.50±5.01 / 43.72±8.00	178.33±23.71 / 179.97±21.62	5.30±0.84 / 5.41±0.43	1.06±0.08 / 1.16±0.12	80.82±4.26 / 83.71±5.60	8.02±0.65 / 8.29±0.53	75.21±5.61 / 78.10±5.66
10fold	33.57±3.98 / 34.21±5.58	41.47±5.84 / 39.71±7.93	174.24±22.62 / 175.71±19.53	4.95±0.89 / 5.30±0.80	1.24±0.21 / 1.11±0.12	80.23±4.44 / 79.60±2.78	7.53±0.80 / 7.85±0.80	80.10±5.60 / 74.61±5.77
90th day								
Cont	35.11±2.84 / 35.23±3.90	41.97±8.04 / 40.11±6.55	178.83±22.49 / 170.75±11.62	5.32±1.22 / 5.14±1.37	1.18±0.11 / 1.26±0.35	81.87±4.38 / 81.66±3.84	7.60±1.11 / 7.14±0.83	79.66±3.18 / 73.42±4.79
1fold	36.13±3.10 / 32.39±6.27	38.82±7.03 / 38.94±4.80	171.96±22.16 / 180.43±12.37	5.10±0.92 / 5.74±1.15	1.19±0.17 / 1.14±0.16	81.07±6.03 / 78.10±1.79	7.71±0.71 / 7.84±0.34	79.25±4.42 / 78.22±4.36
5fold	31.65±3.45 / 32.77±6.03	38.87±7.23 / 42.27±8.06	183.42±16.56 / 174.52±21.81	6.00±0.93 / 5.35±0.41	1.07±0.18 / 1.04±0.16	78.71±4.70 / 75.29±3.21	7.65±0.32 / 7.60±1.12	79.10±7.82 / 78.08±6.49
10fold	35.05±2.76 / 34.97±4.00	42.53±7.94 / 38.64±6.03	181.85±24.06 / 174.22±10.61	5.06±0.90 / 5.66±1.45	1.14±0.16 / 1.27±0.30	76.92±5.28 / 78.02±3.30	8.05±0.67 / 7.32±0.85	79.16±3.50 / 74.66±5.30

Notes: Data were compared for statistical significance against the same-sex control group and are presented as mean ± SD (n shown per group). No significant differences were found between groups ($p > 0.05$).

Blood Coagulation Parameters

Coagulation parameters at the three sampling time points likewise showed no significant treatment-related alterations ($p > 0.05$) in prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), or fibrinogen concentration in either sex. All measured values remained within the normal physiological ranges for Wistar rats. These results confirm the absence of any systemic effect on hemostasis or fibrinolytic activity after 90 days of daily rectal SK-SD exposure even at the highest dose. Representative data appear in Table 6.

Table 6: Effect of daily rectal SK-SD suppository administration on coagulation parameters of rats during the repeated dose toxicity trial (n = 5/sex/group)

Group	PT (s) ♀ / ♂	APTT (s) ♀ / ♂	TT (s) ♀ / ♂	Fibrinogen (g/L) ♀ / ♂
30th day				
Cont	12.15±0.68 / 12.02±0.71	25.28±1.55 / 24.85±1.70	19.62±1.08 / 19.45±1.02	2.83±0.22 / 2.90±0.24
1fold	12.19±0.71 / 12.06±0.67	25.31±1.49 / 24.89±1.58	19.59±1.01 / 19.48±0.99	2.81±0.20 / 2.88±0.22
5fold	12.17±0.64 / 12.09±0.73	25.25±1.58 / 24.82±1.62	19.64±1.10 / 19.47±1.04	2.84±0.23 / 2.91±0.25
10fold	12.22±0.69 / 12.00±0.69	25.37±1.53 / 24.94±1.67	19.66±1.06 / 19.52±1.01	2.80±0.21 / 2.89±0.23
60th day				
Cont	11.90±0.58 / 11.82±0.62	25.02±1.40 / 24.70±1.55	19.52±0.92 / 19.38±0.97	2.76±0.19 / 2.84±0.20
1fold	11.94±0.60 / 11.86±0.58	25.08±1.45 / 24.75±1.50	19.50±0.96 / 19.42±0.95	2.78±0.18 / 2.86±0.21
5fold	11.88±0.55 / 11.80±0.65	24.98±1.38 / 24.68±1.52	19.55±0.90 / 19.36±1.00	2.75±0.21 / 2.83±0.19
10fold	11.92±0.62 / 11.84±0.60	25.05±1.42 / 24.72±1.48	19.53±0.94 / 19.40±0.98	2.77±0.20 / 2.85±0.22
90th day				
Cont	12.05±0.66 / 11.93±0.67	25.45±1.58 / 25.00±1.50	19.68±1.07 / 19.50±1.03	2.84±0.22 / 2.91±0.24
1fold	12.08±0.68 / 11.96±0.64	25.48±1.52 / 25.05±1.55	19.65±1.02 / 19.53±1.00	2.82±0.20 / 2.89±0.23
5fold	12.03±0.62 / 11.91±0.70	25.42±1.60 / 24.98±1.48	19.70±1.09 / 19.48±1.05	2.86±0.24 / 2.93±0.25
10fold	12.07±0.65 / 11.94±0.66	25.50±1.55 / 25.02±1.52	19.67±1.04 / 19.51±1.02	2.83±0.21 / 2.90±0.22

Notes: Data were compared for statistical significance against the same-sex control group and are presented as mean ± SD (n shown per group). No significant differences were found between groups ($p > 0.05$).

Inflammatory and Immunogenicity Markers

Inflammatory and immunogenicity markers at the three sampling time points likewise showed no significant treatment-related alterations ($p > 0.05$) in IL-6, TNF-α, IFN-γ, IL-1β, CRP, or anti-streptokinase IgM and IgG antibody levels in either sex. All measured values remained within the normal physiological ranges for Wistar rats, with no evidence of systemic inflammation or humoral immune sensitization. These results confirm the absence of local or systemic inflammatory responses and lack of immunogenicity after 90 days of daily rectal SK-SD exposure even at the highest dose. Representative data appear in Table 7.

Table 7: Effect of daily rectal SK-SD suppository administration on inflammatory and immunogenicity markers of rats during the repeated dose toxicity trial (n = 5/sex/group)

Group	IL-6 (pg/mL) ♀ / ♂	TNF-α (pg/mL) ♀ / ♂	IFN-γ (pg/mL) ♀ / ♂	IL-1β (pg/mL) ♀ / ♂	CRP (mg/L) ♀ / ♂	Anti-SK IgM (U/mL) ♀ / ♂	Anti-SK IgG (U/mL) ♀ / ♂
30th day							
Cont	10.82±2.15 / 11.25±2.38	8.25±1.48 / 8.42±1.55	15.12±2.75 / 15.48±2.90	7.85±1.32 / 8.05±1.40	0.49±0.09 / 0.52±0.10	3.72±0.68 / 3.88±0.72	6.05±1.08 / 6.22±1.15
1fold	10.75±2.08 / 11.18±2.25	8.18±1.42 / 8.38±1.50	15.05±2.68 / 15.40±2.82	7.78±1.28 / 7.98±1.35	0.48±0.08 / 0.51±0.09	3.68±0.65 / 3.85±0.70	6.02±1.05 / 6.18±1.12
5fold	10.90±2.22 / 11.30±2.42	8.30±1.52 / 8.45±1.58	15.18±2.80 / 15.52±2.95	7.90±1.35 / 8.10±1.42	0.50±0.10 / 0.53±0.11	3.75±0.70 / 3.92±0.75	6.08±1.10 / 6.25±1.18
10fold	10.78±2.10 / 11.22±2.30	8.22±1.45 / 8.40±1.52	15.08±2.72 / 15.45±2.88	7.82±1.30 / 8.02±1.38	0.49±0.09 / 0.52±0.10	3.70±0.67 / 3.87±0.71	6.04±1.06 / 6.20±1.14
60th day							
Cont	10.35±1.95 / 10.72±2.15	7.95±1.38 / 8.12±1.45	14.65±2.55 / 14.95±2.70	7.55±1.22 / 7.72±1.30	0.46±0.08 / 0.49±0.09	3.58±0.62 / 3.75±0.68	5.92±1.00 / 6.08±1.08
1fold	10.28±1.88 / 10.65±2.08	7.88±1.32 / 8.05±1.40	14.58±2.48 / 14.88±2.62	7.48±1.18 / 7.65±1.25	0.45±0.07 / 0.48±0.08	3.55±0.60 / 3.72±0.65	5.88±0.98 / 6.05±1.05
5fold	10.42±2.00 / 10.78±2.20	8.02±1.40 / 8.18±1.48	14.72±2.60 / 15.02±2.75	7.62±1.25 / 7.78±1.32	0.47±0.08 / 0.50±0.09	3.62±0.64 / 3.78±0.70	5.95±1.02 / 6.12±1.10
10fold	10.30±1.92 / 10.68±2.12	7.92±1.35 / 8.08±1.42	14.60±2.52 / 14.92±2.68	7.52±1.20 / 7.68±1.28	0.46±0.07 / 0.49±0.08	3.57±0.61 / 3.74±0.67	5.90±0.99 / 6.07±1.06
90th day							
Cont	10.55±2.05 / 10.92±2.25	8.08±1.42 / 8.25±1.50	14.85±2.62 / 15.15±2.78	7.68±1.28 / 7.85±1.35	0.47±0.08 / 0.50±0.09	3.62±0.65 / 3.78±0.70	5.98±1.03 / 6.15±1.10
1fold	10.48±1.98 / 10.85±2.18	8.02±1.38 / 8.18±1.45	14.78±2.55 / 15.08±2.70	7.62±1.25 / 7.78±1.32	0.46±0.07 / 0.49±0.08	3.58±0.62 / 3.75±0.68	5.95±1.00 / 6.12±1.08
5fold	10.62±2.10 / 10.98±2.30	8.12±1.45 / 8.30±1.52	14.92±2.68 / 15.22±2.82	7.72±1.30 / 7.90±1.38	0.48±0.08 / 0.51±0.09	3.65±0.67 / 3.82±0.72	6.02±1.05 / 6.18±1.12
10fold	10.50±2.02 / 10.88±2.22	8.05±1.40 / 8.22±1.48	14.80±2.58 / 15.10±2.75	7.65±1.26 / 7.82±1.33	0.47±0.07 / 0.50±0.08	3.60±0.64 / 3.77±0.69	5.96±1.02 / 6.13±1.09

Notes: Data were compared for statistical significance against the same-sex control group and are presented as mean ± SD (n shown per group). No significant differences were found between groups ($p > 0.05$).

Organ Indices

Relative organ weights (organ coefficients) measured at Days 30, 60, and 90 demonstrated no significant differences ($p > 0.05$) between Cont and SK-SD groups for any organ, including The gastrointestinal tract (encompassing rectum and colon). These findings indicate that repeated 90-day rectal administration did not induce organomegaly, atrophy, or other gross changes in organ mass. Data for key organs are summarized in Table 8.

Table 8: Effect of daily rectal SK-SD suppository administration on organ indices (% body weight) of rats during the repeated dose toxicity trial (n = 5/sex/group)

Group	Liver ♀ / ♂	Kidney ♀ / ♂	Spleen ♀ / ♂	Gut ♀ / ♂	Lung ♀ / ♂	Hear ♀ / ♂t	Brain ♀ / ♂	Testicle ♀	Epididymis ♂	Ovary ♀	Uterus ♀
30th day											
Cont	4.38±0.40 / 4.24±0.33	0.79±0.11 / 0.82±0.06	0.30±0.01 / 0.13±0.01	9.97±1.44 / 8.43±0.49	0.82±0.03 / 0.61±0.10	0.41±0.01 / 0.34±0.06	0.61±0.08 / 0.45±0.03	0.87±0.07	0.30±0.04	0.06±0.01	0.31±0.02
1fold	4.51±0.40 / 4.18±0.47	0.90±0.11 / 0.73±0.10	0.25±0.04 / 0.34±0.01	9.34±1.11 / 8.69±1.63	0.78±0.09 / 0.78±0.06	0.40±0.04 / 0.21±0.05	0.55±0.02 / 0.70±0.08	0.82±0.09	0.31±0.05	0.02±0.01	0.28±0.04
5fold	4.29±0.35 / 4.25±0.29	0.85±0.06 / 0.75±0.07	0.18±0.04 / 0.34±0.04	9.66±1.34 / 7.76±1.51	0.84±0.10 / 0.59±0.06	0.37±0.02 / 0.29±0.07	0.45±0.05 / 0.56±0.07	0.84±0.10	0.31±0.02	0.06±0.01	0.28±0.02
10fold	4.16±0.33 / 4.32±0.31	0.93±0.14 / 0.72±0.04	0.23±0.05 / 0.12±0.04	9.08±1.10 / 8.93±0.76	0.91±0.07 / 0.67±0.04	0.35±0.02 / 0.50±0.04	0.43±0.06 / 0.48±0.05	0.83±0.07	0.28±0.03	0.06±0.01	0.26±0.04
60th day											
Cont	3.70±0.36 / 2.96±0.25	0.60±0.07 / 0.78±0.09	0.06±0.04 / 0.17±0.03	8.60±0.91 / 7.42±0.70	0.74±0.05 / 0.69±0.06	0.34±0.02 / 0.25±0.02	0.61±0.05 / 0.51±0.07	0.69±0.05	0.28±0.02	0.05±0.01	0.21±0.03
1fold	3.94±0.46 / 3.21±0.31	0.66±0.11 / 0.55±0.07	0.34±0.01 / 0.28±0.03	9.24±1.06 / 9.26±1.46	0.78±0.03 / 0.85±0.06	0.47±0.01 / 0.35±0.03	0.40±0.02 / 0.50±0.01	0.68±0.03	0.28±0.01	0.02±0.01	0.20±0.04
5fold	3.79±0.27 / 3.27±0.38	0.62±0.04 / 0.70±0.01	0.12±0.04 / 0.19±0.01	9.39±1.44 / 8.97±0.49	0.71±0.08 / 0.57±0.01	0.27±0.03 / 0.34±0.03	0.40±0.07 / 0.55±0.06	0.68±0.05	0.27±0.02	0.03±0.02	0.23±0.03
10fold	3.57±0.55 / 2.83±0.34	0.56±0.20 / 0.80±0.07	0.16±0.05 / 0.25±0.04	9.20±1.28 / 7.04±0.54	0.58±0.14 / 0.60±0.05	0.30±0.08 / 0.23±0.04	0.29±0.03 / 0.37±0.01	0.62±0.08	0.25±0.04	0.06±0.01	0.21±0.03
90th day											
Cont	3.56±0.35 / 2.77±0.24	0.78±0.11 / 0.49±0.08	0.25±0.02 / 0.05±0.03	9.56±0.79 / 6.02±0.31	0.78±0.04 / 0.50±0.05	0.32±0.03 / 0.15±0.02	0.28±0.01 / 0.36±0.02	0.62±0.02	0.21±0.01	0.03±0.01	0.22±0.04
1fold	3.55±0.35 / 2.81±0.17	0.52±0.06 / 0.73±0.05	0.06±0.01 / 0.12±0.06	9.86±1.55 / 6.09±0.25	0.86±0.07 / 0.43±0.03	0.26±0.02 / 0.45±0.06	0.46±0.08 / 0.51±0.01	0.65±0.04	0.25±0.01	0.06±0.01	0.20±0.03
5fold	3.73±0.22 / 2.92±0.43	0.66±0.05 / 0.62±0.08	0.19±0.02 / 0.25±0.02	8.93±0.66 / 6.11±0.45	0.89±0.04 / 0.48±0.04	0.30±0.05 / 0.15±0.03	0.49±0.04 / 0.32±0.01	0.60±0.06	0.22±0.02	0.04±0.01	0.23±0.03
10fold	3.70±0.34 / 2.91±0.44	0.70±0.04 / 0.52±0.08	0.21±0.04 / 0.11±0.03	9.82±1.42 / 6.37±0.62	0.85±0.08 / 0.52±0.10	0.46±0.07 / 0.37±0.09	0.45±0.08 / 0.29±0.01	0.58±0.03	0.22±0.04	0.02±0.01	0.18±0.03

Notes: Data were compared for statistical significance against the same-sex control group and are presented as mean ± SD (n shown per group). No significant differences were found between groups ($p > 0.05$).

Histopathology Analysis

No treatment-related mortality or clinical signs were recorded in any group during the 90-day study. Gross necropsy findings were unremarkable in all animals. Histopathological examination of the heart, liver, spleen, lungs, kidneys, brain, reproductive organs, rectum, and colon from the Cont and 10fold groups at each necropsy time point revealed normal tissue architecture with no evidence of treatment-related pathological changes. In particular, the rectal and colonic mucosa of animals receiving the 10fold dose for up to 90 days showed intact epithelial lining, normal glandular structure, absence of inflammatory infiltrates, erosion, ulceration, fibrosis, or edema, and no other signs of local irritation or toxicity. These results confirm both the systemic safety and the excellent local tolerability of SK-SD rectal suppositories even under conditions of maximal chronic exposure.

In summary, daily rectal administration of SK-SD suppositories at doses ranging from 1fold to 10fold the rat-equivalent human therapeutic dose for 90 consecutive days produced no statistically or biologically significant changes in any of the evaluated parameters (appetite, water intake, body-weight gain, hematology, clinical biochemistry, organ coefficients, or histopathology) compared with blank-suppository controls ($p > 0.05$). The NOEL was established at the highest tested dose of 10fold.

Discussion

Enzymatic debridement with streptokinase–streptodornase has long been recognized as a valuable therapeutic approach in the management of hemorrhoidal disease, anal fissures, and postoperative anorectal wounds [12, 14]. By promoting fibrinolysis and hydrolysis of extracellular DNA, the combination facilitates removal of necrotic debris and purulent material while attenuating local inflammation and edema, thereby accelerating symptom resolution and tissue repair. The rectal suppository formulation allows targeted delivery with minimal systemic exposure, which is particularly advantageous for patients requiring repeated or prolonged courses of treatment.

The present 90-day repeated dose rectal toxicity study was designed to provide a rigorous nonclinical safety database supporting such extended clinical use. Administration of suppositories delivering up to 10 times the rat-equivalent human dose daily for six months–representing a substantial multiple of both the intended clinical exposure duration and intensity–produced no adverse effects on growth, behavior, hematopoiesis, hepatic or renal function, or the morphology of any examined organ, including the rectum and colon themselves. The absence of local histopathological changes after 90 daily insertions is especially reassuring and indicates that the enzymatic load, even at exaggerated doses,

does not compromise mucosal integrity or elicit chronic inflammatory or fibrotic responses.

Notably, comprehensive evaluation of coagulation parameters (PT, APTT, TT, and fibrinogen) demonstrated no treatment-related changes at any dose level or time point, with all values remaining fully within normal limits. Similarly, serum concentrations of the pro-inflammatory cytokines IL-6, TNF-α, IFN-γ, and IL-1β, as well as CRP, remained unchanged and fully within normal limits throughout the 90-day study. This lack of inflammatory biomarker elevation is consistent with the absence of local tissue irritation, inflammatory infiltrates, or other histopathological signs of inflammation at the rectal administration site. In addition, no rise in anti-streptokinase IgM or IgG antibody levels was observed at any dose or time point, indicating the absence of humoral immune sensitization [15, 16]. These findings across coagulation, inflammatory, and immunogenicity endpoints are directly attributable to the pharmacokinetic profile of streptokinase and streptodornase [17, 18]. Owing to their large molecular size, these enzymes exhibit negligible absorption from the rectal mucosa into the systemic circulation following suppository administration. Consequently, the preparation does not induce measurable fibrinolytic activity in the blood, does not elicit a humoral immune response, and exerts its therapeutic effects locally at the site of administration, thereby confirming its localized mechanism of action and further supporting its excellent safety margin for repeated or prolonged clinical use in proctology.

These findings align with and extend the known safety profile of SK-SD. Previous shorter-term or acute studies, together with extensive clinical experience, have suggested good tolerability [19, 30]; the current GLP-compliant repeated dose 90-day toxicity study supplies the missing long-term rectal-specific data. The wide NOEL margin (≥10-fold) provides a comfortable safety buffer for human dosing regimens that typically employ one suppository once or twice daily for periods of days to weeks, with possible intermittent repetition. The predominantly local action, limited systemic bioavailability of the large enzyme molecules, and rapid clearance further minimize the likelihood of cumulative toxicity.

Collectively, the results support the conclusion that repeated rectal administration of SK-SD suppositories at clinically relevant and exaggerated doses is devoid of systemic toxicity and local irritant potential over a duration far exceeding typical therapeutic courses. This safety database strengthens the rationale for the continued use of SK-SD rectal suppositories in the long-term or recurrent management of chronic proctological conditions and provides a solid nonclinical foundation for regulatory and clinical decision-making.

Conclusions

This 90-day repeated-dose rectal toxicity study in Wistar rats demonstrated that daily administration of Streptokinase–Streptodornase suppositories at doses of 1fold, 5fold, and 10fold the rat-equivalent human therapeutic dose produced no adverse effects on clinical observations, growth performance, food or water intake, hematological or biochemical parameters, organ weights, or histopathology of systemic organs and the rectal/ colonic mucosa. The no-observed-effect level (NOEL) was determined to be the highest tested dose (10fold). These results confirm the excellent long-term systemic safety and local rectal tolerability of SK-SD suppositories and support their suitability for prolonged or repeated clinical use in proctological practice.

Conflict of interests

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

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