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OECD GUIDED- REPEATED DOSE 28-DAY ORAL TOXICITY STUDY OF THE NEWLY FORMULATED VALACYCLOVIR MICROBEADS

Sankar Narayan Bhunia[1,2#], Dipankar Saha[1#], Sudipta Das[2*], Trishna Das[1], Sawan Das[2], Piyali Devroy[3], Bishal Saha[3], Pallab Kanti Haldar[4], Asis Bala[3]

[1]School of Pharmacy, Girijananda Chowdhury University, Guwahati, Assam, 781017, India.
[2]Department of Pharmaceutics, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, 741222, Nadia, West Bengal, India.
[3]Institute of Advanced Study in Science and Technology (IASST), Vigyan Path, Guwahati, PIN- 781035 Assam, India.
[4]School of Natural Product Studies (SNPS), Jadavpur University (JU), Kolkata, West Bengal, India 700032.

***Corresponding Author: Dr. Sudipta Das**
Department of Pharmaceutics, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, 741222, Nadia, West Bengal, India.

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Joint First author: Sankar Narayan Bhunia, Dipankar Saha

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ABSTRACT

The study aimed to evaluate the oral toxicity of a newly formulated valacyclovir microbeads, following OECD-guided, repeated-dose, 28-day testing protocols. The experiment was conducted in accordance with OECD 407 guidelines for acute oral toxicity. A total of 24 healthy young adult Wistar rats were divided into four groups (n=6; Male: Female = 1:1). The groups were treated as follows: a control group and three dose groups receiving low, medium, and high doses of 100, 200, and 400 mg/kg body weight, respectively, daily for 28 days. No significant differences were observed in terms of body weight changes, food consumption, or histological analysis of liver and kidney tissues. Furthermore, there was no hematological difference between the treated and untreated groups. These findings confirm the safety of the newly formulated valacyclovir drug.

KEYWORDS: Valacyclovir microbeads; adult Wistar rats: 28-day repeated Toxicity Study.

INTRODUCTION

Controlled drug delivery systems have significantly improved pharmaceutical therapy by increasing the precise, controlled, and sustained release of therapeutic agents, thereby addressing many of the drawbacks associated with conventional dosage forms.[1] Among the various delivery technologies developed over the years, microbead-based systems have obtained significant attention due to their versatility and therapeutic potential.[2] Microbeads are small, free-flowing, spherical particles, generally ranging from 1 to 1000µm in diameter, composed of a drug-loaded polymeric matrix designed to regulate both the rate and location of drug release.[3] Owing to their capacity to improve drug bioavailability, extend therapeutic action, and minimize dosing frequency, microbeads have now become an important shift in pharmaceutical research and formulation development.

Within this advanced category, hydrogel-based microbeads have gained significant attention for their distinctive physicochemical properties. Characterized by high water content, a modifiable three-dimensional polymeric architecture, and favorable biocompatibility, hydrogel matrices are uniquely fit for the encapsulation of a wide range of therapeutics. Drug release from these systems is primarily driven by swelling-mediated diffusion, a mechanism that can be fine-tuned by carefully selecting polymer composition, crosslinking density, and bead morphology. These physicochemical properties have made hydrogel microbeads highly adaptable platforms for controlled, site-specific, and long-lasting drug delivery.[4]

Despite advances in formulation science, many clinically important drugs pose pharmacokinetic challenges that limit their therapeutic potential. Valacyclovir hydrochloride, a well-established antiviral prodrug, is a

compelling example of this class. As a prodrug of acyclovir, valacyclovir is rapidly hydrolyzed following oral administration by intestinal and hepatic valacyclovirase enzymes to yield its pharmacologically active form, which inhibits viral DNA polymerase and disrupts the replication of herpes simplex virus (HSV) and varicella-zoster virus (VZV). While this enzymatic conversion confers superior oral bioavailability compared to acyclovir itself (approximately 55%), the drug nonetheless suffers from a short biological half-life of 2.5 to 3 hours.[5] This pharmacokinetic profile necessitates multiple daily administrations to endure therapeutic plasma concentrations, a requirement that contributes to poor patient adherence, pronounced peak-trough fluctuations, and inconsistent clinical outcomes, particularly in patients undergoing long-term antiviral prophylaxis.[6]

A sustained-release microbead formulation of Valacyclovir can help overcome the limitations of conventional therapy by maintaining therapeutic drug levels for longer periods and reducing dosing frequency. Sodium alginate forms stable hydrogel matrices via cross-linking with aluminum ions (Al^{3+}), while Gellan gum enhances gel strength and regulates drug release. Together, these polymers can create an effective controlled-release system that minimizes plasma concentration fluctuations, improves patient compliance, and enhances the management of chronic herpesvirus infections.[4] This article presents the development and in vivo safety evaluation of hydrogel-based microbeads for the sustained delivery of valacyclovir hydrochloride. To assess the systemic tolerability of the formulation, a 28-day repeated-dose oral toxicity study was conducted in Wistar rats in accordance with OECD Guideline 407. Animals of both sexes received escalating doses of 100, 200, and 400 mg/kg body weight per day over the stipulated study period, with comprehensive monitoring of mortality, clinical signs, body weight, food consumption, neurobehavioral parameters, hematological indices, organ weights, and histopathological findings. The findings of this evaluation are expected to provide a robust preclinical safety profile to support the formulation's further development toward clinical application.

MATERIALS AND METHODS

Formulation of the drug: The hydrogel-based microbead formulation of Valacyclovir Hydrochloride is reported by Saha et al. (2025). The drug was formulated, and its physical and chemical parameters were tested before the paper mentioned.

Animals: Healthy young adult Wistar rats, both male and female, were used for the study. A total of 24 animals were grouped into four groups (n=6; Male: Female= 1:1. The rats were housed under standard laboratory conditions, with a temperature of 21 ± 3°C, relative humidity of 30–70%, and a 12-hour light/dark cycle, along with ad libitum access to food and water.

Experiment Design: The animals were divided into different groups: a normal control group, a vehicle control group, and three treatment groups. Each group comprised an equal number of males and females (1:1 ratio). After a minimum acclimatization period of 5 days, the test item was administered once daily via oral gavage using an appropriate cannula attached to a graduated syringe. The maximum volume used is 1ml/kg b.w. The study was approved by the IAEC of IASST vide IAEC Approval Number: IASST/IAEC/2026/16/08. The ARRIVE regulatory guidelines are strictly followed during the experiment and documentation.

Repeated Dose 28-day oral toxicity study: The experiment was conducted in accordance with the OECD 407 Acute oral toxicity guidelines. 24 animals were divided into 3 test groups that received 100, 200, and 400 mg/kg b.w. A normal group and a vehicle control group were kept and received. A total of 4 groups were observed during the 28-day toxicity study. Observations included twice-daily checks for mortality, daily monitoring of clinical signs, weekly assessments of body weight and feed consumption, and detailed clinical examinations. During the final week of the study, neurobehavioral assessments, including functional observations, were conducted.

Pathological Studies: At the 28^{th} day, the rats were starved and anesthetized using Isoflurane. Blood collected from cardiac puncture and kept in EDTA tubes from every group was used to perform hematological analysis. Different organs like the kidney, liver, heart and lungs were collected in normal saline and 10%(v/v) formaldehyde. Further histopathological and biochemical studies of the collected organs were performed.

Statistical Analysis: Data were presented graphically with SEM around the Mean. The data from the 28-day repeated oral toxicity study were analyzed using ANOVA, with p-values ≤ 0.05 considered statistically significant.

RESULTS

Clinical Observations: Throughout the study, no treatment-related mortality was reported in any group. Clinical evaluations consistently indicated that all animals showed no abnormal signs; they remained healthy and active throughout the study period. The drug formulation administered did not induce any observable changes in behavior, and no deviations in behavioral patterns, motor activity, grip strength, or sensory responses were observed among all dosage groups compared with the control group. Overall, the findings suggested a well-tolerated treatment with no adverse effects on the animals' health or behavior.

Body Weight: Body weights were recorded daily for each animal across all groups, providing a clear overview of their growth trajectories. The measurements revealed a consistent and typical growth pattern in both male and

female subjects within each group. Notably, there were no statistically significant differences in body weight between the control and treated groups, suggesting that the treatments did not adversely affect growth (Figure 1).

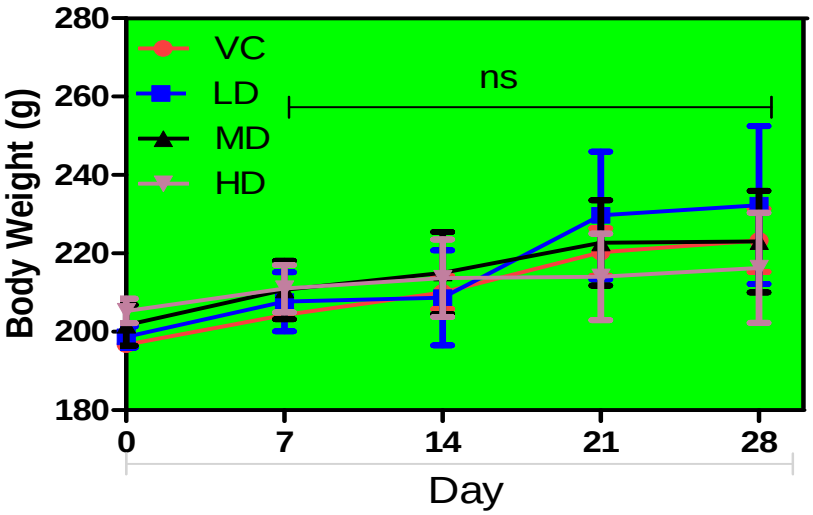

Fig 1: Graphical representation of day-wise body weight differences in different groups after performing ANNOVA. VC- Vehicle Control, LD – Low Dose, MD – Medium Dose, and HD- High Dose.

Food Consumption: Daily, each animal was supplied with a measured 50-gram portion of food. After a full 24-hour period, the amount of food consumed was evaluated by weighing the leftover food. Throughout the study, food consumption levels were consistent across all groups, and no significant treatment-related variations were observed at any point during the observation period (Figure 2).

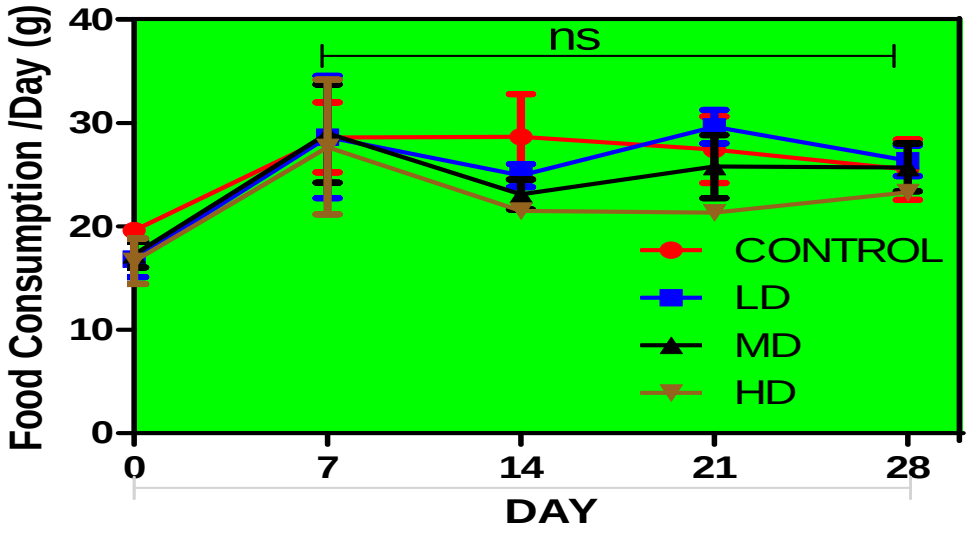

Fig 2: Graphical representation of day-wise food consumption relation in different groups after performing ANOVA. Control, LD – Low Dose, MD – Medium Dose and HD- High Dose.

Histopathology of Kidney and Liver: Hematoxylin and eosin (H&E) staining was performed on tissue samples from both the kidney and the liver. The analysis revealed no notable differences in staining characteristics between liver and kidney tissues, suggesting uniform histological appearance across these samples (Figure 03).

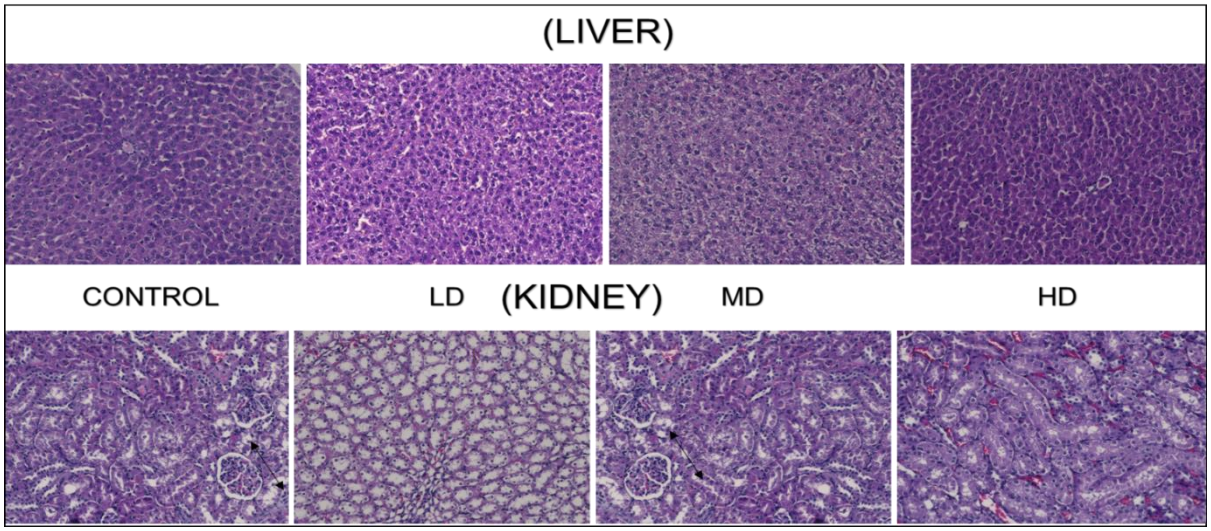

Fig 3 – Liver and Kidney tissue samples of different treatment groups after H&E staining.

Hematological Analysis: Blood samples were gathered through cardiac puncture, a precise method for obtaining samples directly from the heart. Analysis of red blood cells (RBC), white blood cells (WBC), and hemoglobin levels was conducted using a semiautomated hematology analyzer, which provides accurate, efficient results. Upon examination, no notable differences were observed in any of the three hematological parameters between animals that received treatment and those that remained untreated. This suggests that the treatment did not have a significant impact on these specific blood characteristics (Table 1).

Table 1: Hematological analysis of the blood collected from experimental animals. No statistical significance found between the groups.

Group	RBC (10^6/µL)	Hb g/dL	WBC 10^3/µL
Control	8.427±0.54	13.84±1.32	13.89±1.01
LD	8.31±0.58	14.43±1.11	12.15±1.12
HD	8.45 ±1.01	14.190±1.09	12.49±0.98
HD	8.34±0.98	14.45±0.98	12.89±1.5

DISCUSSION

Direct toxicity testing of xenobiotics in humans would provide the most relevant data; however, such studies are ethically unacceptable.[7] Consequently, toxicity assessments are typically conducted using animal models, with rats being among the most commonly employed. Rats are widely preferred because they share several physiological and metabolic characteristics with humans, while their small size, short lifespan, and rapid reproductive cycle make them practical and cost-effective for experimental research.[8]

In this study, the toxicity of a Valacyclovir microbead formulation was assessed. This study showed no significant toxicity in rat models even at high doses. Food consumption, body weight differences, and other clinical observations, including mortality and behavioral changes, were not significantly affected at any treatment level. The biochemical and pathological study of blood showed no distinct toxicity of the compound. Histopathological examination of kidney and liver tissue samples showed no significant cell necrosis or apoptosis. Thus, the rats' metabolic activity in the different treatment groups remained normal, with no signs of acute toxicity. Hence, it can be interpreted that the formulated Valacyclovir drug may be safe for oral use.

CONCLUSION

The repeated oral dose of the newly formulated valacyclovir microbeads for 28 days, as per OECD 407, showed no significant toxicity at concentrations up to 400mg/mL. However, the doses should be given with caution when given for an extended period (more than 28 days).

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