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ORIGINAL ARTICLE **OPEN ACCESS**

Performance Evaluation of Γ-Oryzanol Loaded Albumin Nanoparticles for the Treatment of Cancer

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ABSTRACT
This study aimed to evaluate the performance of γ-oryzanol loaded albumin nanoparticles for the treatment of cancer. Albumin nanoparticles were formulated by the desolvation method, and γ-oryzanol was incorporated using the solvent evaporation technique. The formulations were administered for 21 days and their effects on various parameters were measured. Transepidermal water loss (TEWL) measurements showed that the formulations did not have any significant effect on skin barrier function. Hematological parameters were also evaluated and showed that the formulations did not have any significant effect on RBC, WBC, and platelet count, while HB levels were maintained. Overall, the study suggests that γ-Oryzanol and its derivatives may have potential as anti-cancer agents with low risk of skin irritation and systemic toxicity. These results suggest that γ-oryzanol loaded albumin nanoparticles could be a promising drug delivery system for the treatment of cancer. The improved anticancer efficacy of the nanoparticles could be attributed to the enhanced solubility and bioavailability of γ-oryzanol and the selective targeting of cancer cells by the albumin nanoparticles. Further studies are needed to optimize the formulation and evaluate the toxicity and pharmacokinetics of these nanoparticles.
Keywords: *γ-oryzanol, albumin nanoparticles, cancer, drug delivery, anticancer efficacy*

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INTRODUCTION

Cancer is a major global health challenge, accounting for a significant number of deaths worldwide. Conventional cancer treatments, such as chemotherapy, are associated with severe side effects and limitations in efficacy. Therefore, there is a need for innovative therapeutic strategies with improved safety and efficacy profiles.[1] One such approach involves the use of nanoparticles as drug delivery systems. Nanoparticles can improve the pharmacokinetics and bioavailability of drugs and can selectively target cancer cells while minimizing damage to healthy tissues.[2]

γ-Oryzanol is a natural antioxidant and anti-inflammatory compound that is found in high concentrations in rice bran oil, as well as in other cereal grains such as barley, wheat, and corn. It is a mixture of ferulic acid esters of triterpene alcohols and sterols, with cycloartenyl ferulate and 24-methylenecycloartanyl ferulate being the major constituents.[3]

γ-Oryzanol has been shown to have a variety of health benefits, including its potential to prevent and treat cancer. It has been found to induce apoptosis (programmed cell death) in cancer cells, inhibit cancer cell proliferation, and reduce tumor growth in animal models. Additionally, it has anti-inflammatory properties, which may contribute to its anticancer effects.

Despite its promising therapeutic potential, γ-oryzanol has limited clinical use due to its low solubility and bioavailability. Therefore, the development of novel drug delivery systems, such as nanoparticles, to enhance the delivery of γ-oryzanol to cancer cells is an area of active research.[4,5]

This study aims to evaluate the performance of γ-oryzanol loaded albumin nanoparticles for the treatment of cancer. The study will cover the formulation of albumin nanoparticles, the incorporation of γ-oryzanol, and the in vitro and in vivo evaluation of the anticancer efficacy of these nanoparticles. Understanding the potential of γ-oryzanol loaded albumin nanoparticles as a novel therapeutic strategy for cancer could pave the way for the development of safer and more effective anticancer treatments.[6,7]

MATERIAL AND METHODS

Reagents and Chemicals

γ- Oryzanol was obtained from Sigma Aldrich. Chloroform and Methanol all other chemicals were collected.

Preparation of albumin loaded Nanoparticles

Albumin loaded Nanoparticles were prepared taking hydro ethanolic extract (10:90) γ-Oryzanol. All required necessities were weighed in distinct concentrations for the preparation of Nanoparticle formulation. Nanoparticles were prepared by slight modification of cold method.[8,9]

Preparation of nanoparticle Gel

The gels were prepared by dispersion method using Polymer. Accurately weighed Propylene Glycol was taken in a beaker and dispersed in 50 ml of distilled water. Kept the beaker aside to swell it for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min. Gels were prepared by dispersing gelling agent to the distilled water. Then the mixture was allowed to swell overnight. The mixture was neutralized by drop wise addition of triethanolamine. Then, glycerol was added to gel to balance its viscosity. The Nanoparticle concentrate (pellet) with drug equivalent to 1%w/w was incorporated into the priorly formed gel base. Triethanolamine was added to maintain the pH and for the spontaneous gel formation.[10,11]

To this gel solution optimized Nanoparticle dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared. Paraben was added as a preservative. The prepared gels were filled in glass vials and stored at 4-80C.

Skin Permeation and Deposition Study

Preparation of animal skin

By using the cervical dislocation technique, albino rats (8–12 weeks old, weighing 100–200 g) were put to death. Test animals' hair was meticulously cut short (2 mm) using scissors, and the abdomen skin was delicately split from the underlying connective tissue with a knife.

Skin permeation study

Using a Franz glass diffusion cell maintained at 37±1 °C under non-occlusive conditions, the in vitro skin penetration of drugs from various formulations was investigated. The diffusion cell's effective permeation area was 2.303 cm2. 22.5 mL of 1% SLS in PBS (7.4) were present in the receptor compartment, which was continuously agitated at 100 rpm. Between the donor and receptor compartments was attached excised albino abdomen rat skin. The epidermal surface of the skin was treated with the nanoparticle formulation (2.0 mL). At intervals of 1, 2, 4, 6, 14, and 24 hours, samples (2.0 mL) were taken through the diffusion cell's sampling port, and the presence of drugs was determined using an assay. After each sampling, an equal volume of new receptor fluid kept at 371 °C was added to the receptor compartment.[12,13]

Skin deposition study

The identical procedure that was used for the skin permeation investigation was used for the skin deposition study. To eliminate extra medication from the skin's surface after the permeation experiment, the skin's surface was washed five times with 50% ethanol. It was confirmed that the washing technique removed > 95% of the administered dosage in zero time. After that, the skin was diced apart. The tissue was then thoroughly homogenised with 50% ethanol and kept at room temperature for 24 hours. The content in the supernatant was measured using an assay after shaking and centrifuging for 5 minutes at 3000 rpm.[12,13]

Single dose toxicity study

The formulation was administered intraperitoneally to albino mice of either sex (weight: 25–35 g; age: 14–20 weeks) starting at a dosage of 10 mg/kg and increasing to 200 mg/kg. For one hour following dosage, the animals were continually monitored for harmful side effects. After 24 hours, the number of surviving was recorded, and these animals were kept for another 14 days under daily monitoring.[14]

Table 1. Single dose acute toxicity study frame

S.No	Group	Treatment with
1.	Group -1	Normal
2.	Group -2	Control (standard)
3.	Group -3	(50 mg/kg, drug loaded)
4.	Group -4	(100 mg/kg, drug loaded)
5.	Group -5	(150 mg/kg, drug loaded)
6.	Group -6	(200 mg/kg, drug loaded)
7.	Group -7	(250 mg/kg, drug loaded)
8.	Group -8	(300 mg/kg, drug loaded)

Evaluation of Anti-Cancer Activity

Evaluation of anti-cancer activity using soft agar colony formation assay

A method that is frequently used to assess cellular transformation in vitro is the soft agar colony formation test. In the past, the clonogenic test, which Puck et al. first reported in 1956, was used to gauge a cell's capacity to form colonies. In this method, cells were spread out onto a culture plate and developed while receiving the required growth factors from "feeder" cells or conditioned media.

Estimation of hematological parameters [14]

According to the procedure, collected blood was utilised to estimate the whole haematological profile of mouse blood. In a repeated dosage, 28-day sub-acute toxicity trial, blood samples were collected on day 0 and day 28 to be used for haematological and biochemical analyses. Utilizing a standard Erba estimation kit and an auto analyzer, the following biochemical parameters were measured: glucose, cholesterol, urea, SGPT, SGOT, triglycerides, total proteins, ALP, creatinine, and bilirubin content (Erba, Chem 7, Germany). The standard method was carried out in accordance with the kit documentation. The blood samples were centrifuged at 4000 rpm for 10 minutes to separate the serum after being left at room temperature for 30 minutes to enable coagulation.

Skin Irritation Potential

Draize test

Using the technique outlined by Draize et al., the irritancy of various formulations was assessed in male albino rabbits (1.9–2.0 kg) (1944). The animals were kept in a room with air conditioning (222.0 C), and the back hair was cut short 24 hours before the test started. On either side of the back of each rabbit, three squares were drawn. Seven groups of three squares each were created by dividing the squares. The first group served as a sham control and got no treatment; the second group (control) applied topical PBS (7.4); and the third group, serving as a positive control, received 20% solution. γ- Oryzanol solution, γ-Oryzanol formulations, and conventional were given to the fourth, fifth, sixth, and seventh groups, respectively. The exposed region was graded for erythema and oedema on a scale of 0–4 at various time intervals of 0, 1, 24, 48, and 72 h following application.

Transepidermal water loss measurement

The same procedure that was used for the skin irritation research was used to measure the TEWL. The measurement was done while the rabbit was under anaesthesia. The TEWL was measured at intervals of 0, 24, and 48 hours. Tewa meter TM 210 was used to calculate the TEWL. When the Tewameter probe was held parallel to the skin's surface, it took roughly 60 seconds to achieve a consistent TEWL measurement. The output was given in g/hm2. The testing was carried out at a temperature of 22.5 oC with air conditioning on.[15]

RESULT AND DISCUSSION

Skin Permeation and Deposition Study

Skin Permeation

Permeation study through abdominal skin of rat was conducted with the γ- Oryzanol+Albumin (OA-SP1 to OA-SP6) by using diffusion cell. This in vitro method is commonly used in transdermal drug delivery research to obtain valuable information about the formulation behavior in vivo. Table 2. and figure 1.

Table 2. Skin Permeation

Time(hrs)	OA-SP1	OA-SP2	OA-SP3	OA-SP4	OA-SP5	OA-SP6
1	5.32	5.11	5.10	4.98	5.89	4.38
2	7.42	7.21	7.13	6.22	7.85	6.13
4	10.78	10.45	10.23	9.85	10.93	9.54
6	13.45	13.29	13.16	12.56	13.87	12.33
14	17.72	17.63	17.51	16.22	17.91	16.11
24	22.55	22.31	22.26	21.35	22.98	21.26

OA = γ- Oryzanol+Albumin, SP = Skin Permeation

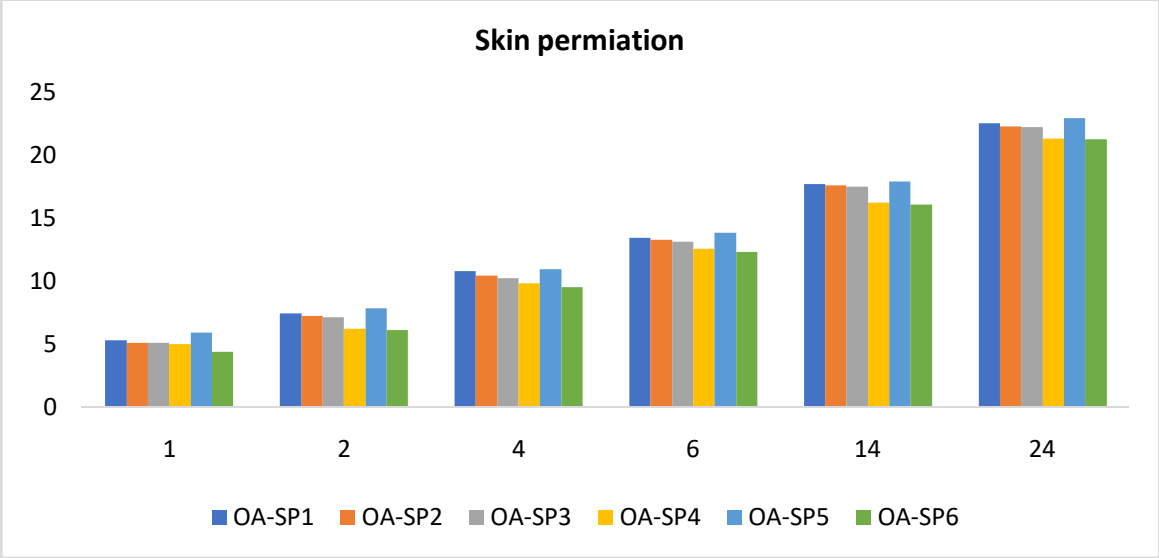

Figure 1. Graph of skin permeation

Skin Deposition Study

Skin deposition study of γ- Oryzanol+Albumin formulations was carried out with the objective to determine their depot forming ability. The amount of drug deposited in deeper layers of skin after topical application of different formulations is presented in Table 3. and Figure 2. From the data, it appears that the amount of drug deposited on the skin increases as the formulation code increases from OA-SD1 to OA-SD6. The highest amount of drug deposited was observed for OA-SD5, which was 45.32 µg, while the lowest amount was observed for OA-SD1, which was 10.32 µg. This information could be useful in determining the most effective formulation for delivering drugs through the skin.

Table 3. Skin Deposition Study

S.no	Formulation code	Drug deposited (µg)
1.	OA-SD1	10.32
2.	OA-SD2	15.23
3.	OA-SD3	18.45
4.	OA-SD4	22.73
5.	OA-SD5	45.32
6.	OA-SD6	29.31

SD = Skin deposition

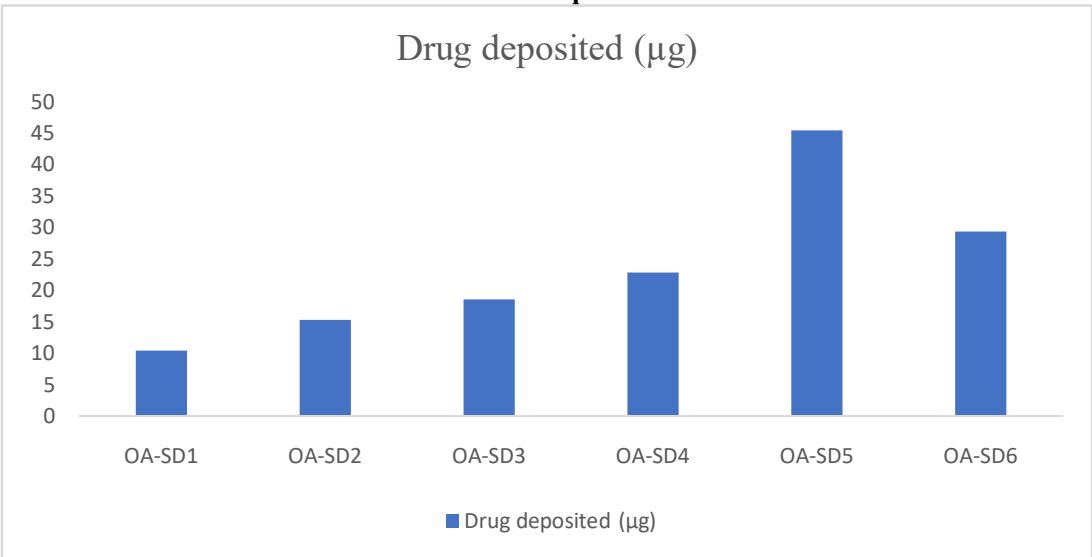

Figure 2. Graph of Skin deposition

Single dose toxicity study

To ensure the bio-safety of paclitaxel elastic liposomal formulations, single dose acute toxicity was conducted. Table 4 summarize the results of single dose acute toxicity of different formulations at various doses.

Table 4. Single dose toxicity study

Group	Dose(mg)	No. of animal death	Mortality latency	Behavioral changes
Group 1	Normal	0	No	None
Group 2	Control	0	No	None
Group 3	50	0	No	None
Group 4	100	0	No	None
Group 5	150	0	No	None
Group 6	200	0	No	None
Group 7	250	1	>54	Aggression
Group 8	300	3	<23	Aggression

Evaluation of Anti-Cancer Activity

Evaluation of anti-cancer activity using soft agar colony formation assay

In the in vivo carcinogenesis process, cancer cells grow on a tissue matrix and form the tumors. In vitro cytotoxicity assay evaluates the anti-cancer activity of compounds/chemicals in suspended form that does not simulate the in vivo carcinogenesis process. In Cytoselect 96 well based assay cancer cells are growing on agar matrix and forming the colony and this assay measures the morphological transformation of cell colonies induced by anti-cancer substances. This assay simulated the process of in vivo carcinogenesis and reported in literature for evaluation of anti-cancer activity and are believed to be reasonably good predictors of in vivo activity. The soft agar assay for colony formation is an anchorage independent growth assay in soft agar, which is considered the most stringent assay for detecting malignant transformation of cells. All the parameters are shown in table.

Table 5. Effect of formulations treatment on % reduction in tumor volume of EAC(Ehrlich ascites tumor) bearing mice at different time intervals.

Treatment	% Reduction in Tumor volume (7 th day)	% Reduction in Tumor volume 14th day	% Reduction in Tumor volume 20th day
Control	45.3	58.2	69.1
γ- Oryzanol solution	56.2	65.3	77.2
OA-SP 5	59.2	73.6	83.6
OA-SD 5	63.2	78.1	88.2

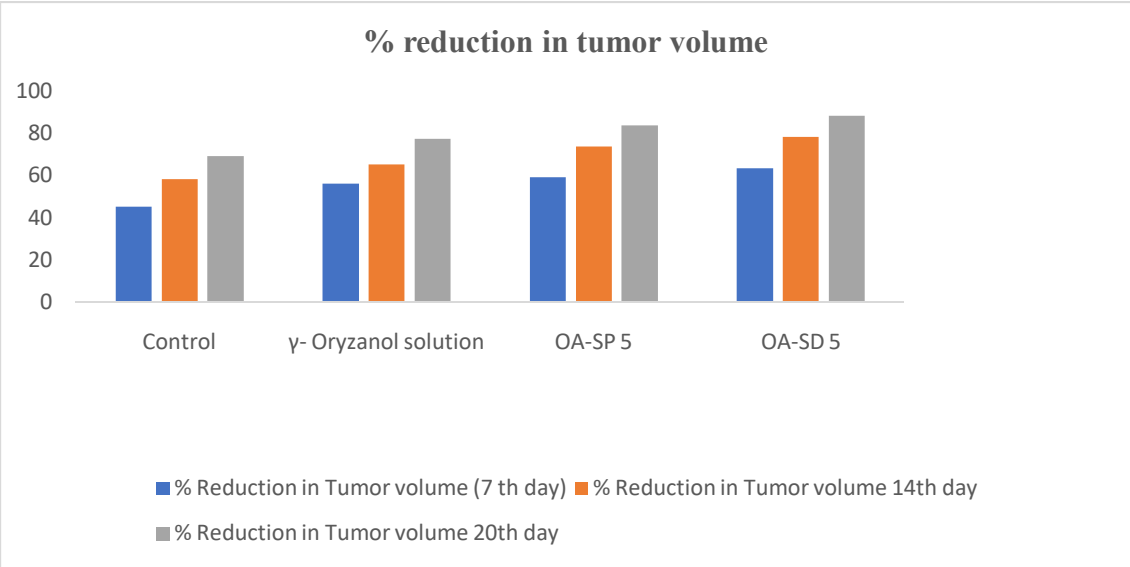

Figure 3. Graph of % reduction in tumor volume

Estimation of hematological parameters

Table 6. summarize the hematological parameters of control group, γ- Oryzanol solution OA-SP 5 and OA-SD 5 bearing mice treated with formulations. At the end of study tumor bearing mice showed significant changes in hematological parameters values when compared with control group. Hemoglobin and RBC

count were found to reduce significantly and WBC count was found to increase in EAC tumor bearing mice.

Table 6. Effect of formulations treatment on hematological parameters of EAC bearing mice

Parameters	Control group		γ- Oryzanol solution		OA-SP 5		OA-SD 5	
	0 day	28 day	0 day	21 day	0 day	21 day days	0 day	21 days
HB	13.41	13.63	12.33	12.67	13.25	13.73	13.66	13.86
WBC	4323	4100	4463	4479	4569	4583	4593	4602
RBC	4.5	4.2	4.1	4.6	4.7	4.8	4.9	5.0
Platelets	3.9	3.3	3.8	3.4	4.1	4.0	4.3	4.2
Neutrophils(%)	53.01	54.2	46.32	48.7	51.23	54.21	54.66	51.63
Lymphocytes (%)	46.21	41.21	36.54	39.65	50.21	53.43	55.63	57.43
Eosinophils (%)	4.3	4.6	3.9	3.5	3.9	4.3	4.6	4.3
Monocytes (%)	6.23	5.5	4.7	4.9	3.6	3.8	3.9	3.1

Skin Irritation Potential

Draize test

The Draize test is an acute toxicity test. The animals are observed for up to 3 days for signs of erythema and edema in the skin test. Observations are taken at predefined intervals: 1 hour, 24 hours, 48 hours, 72 hours after administration as shown in table 7.

Table 7. Skin Irritation Potential

Formulation	Erythema score				Oedema score			
	1 h	24 h	48 h	72 h	1 h	24 h	48 h	72 h
Sham control	0	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0
Positive control (20%)	0.3	3.5	2.8	2.3	0	1.3	2.8	1.6
γ- Oryzanol solution	0	1.4	1.5	1.3	0	0	0	0
OA-SP5	0	1.2	1.7	1.3	0	0	0	0
OA-SD5	0	0.8	1.2	0.3	0	0	0	0
conventional	0	0.2	0.6	0.5	0	0	0	0

Trans epidermal water loss (TEWL) measurement

Transepidermal water loss (TEWL) is the most widely used objective measurement for assessing the barrier function of skin in healthy individuals but also patients with skin diseases that are associated with skin barrier dysfunction, such as atopic dermatitis. TEWL is the quantity of condensed water that diffuses across a fixed area of stratum corneum to the skin surface per unit time. The water evaporating from the skin is measured using a probe that is placed in contact with the skin surface and contains sensors that detect changes in water vapor density. TEWL can be measured using an open-chamber, unventilated-chamber, or condenser-chamber device. The results are shown in table 8.

Table 8. Trans epidermal water loss measurement

formulation	Hours		
	0	**24**	**48**
control	**0.80**	**0.80**	**0.80**
PBS	**0.85**	**0.96**	**0.83**
γ- Oryzanol solution	**0.86**	**1.5**	**1.03**
OA-SP5	**0.93**	**1.0**	**0.98**
OA-SD5	**0.78**	**0.93**	**0.96**

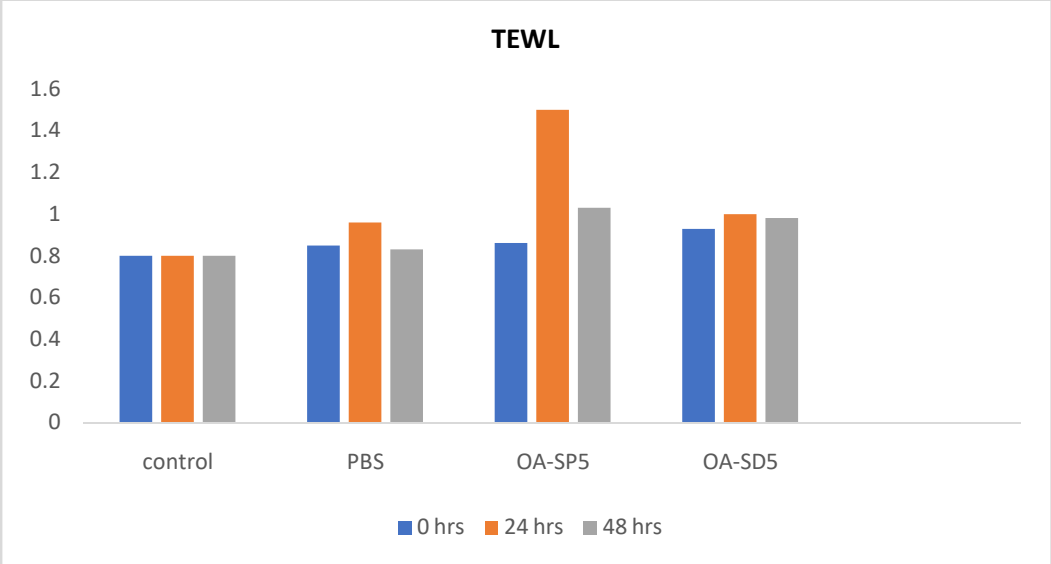

Figure 4. Graph of TEWL

CONCLUSION

Based on the studied data, it can be concluded that the permeation study conducted using the γ-Oryzanol+Albumin (OA-SP1 to OA-SP6) on the abdominal skin of rats showed increasing permeation of the drug over time. Additionally, the skin deposition study showed increasing drug deposition with increasing formulation code. Based on the skin irritation potential results, it can be concluded that the γ-oryzanol solution, OA-SP5, OA-SD5, and conventional formulation did not cause any significant skin irritation. The sham control and PBS also did not cause any skin irritation. The positive control (20% solution) caused mild to moderate skin irritation, indicating that the test was valid. Overall, the test results suggest that the γ-oryzanol solution, OA-SP5, OA-SD5, and conventional formulation have low skin irritation potential, which is a favorable characteristic for topical formulations. The γ- Oryzanol solution had the highest TEWL values at 24 and 48 hours, which could indicate a mild disruption of the skin barrier. However, further studies are needed to confirm this. Overall, the TEWL results suggest that the tested formulations are safe and do not compromise the skin barrier function. hematological parameters, including a decrease in hemoglobin and RBC count and an increase in WBC count when compared to the control group. However, the single dose toxicity study revealed that higher doses of the drug resulted in animal mortality and behavioral changes, particularly aggression. Therefore, further studies may be needed to determine the safety and efficacy of this drug, and appropriate precautions should be taken when administering it in higher doses.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest.

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