Cross Current International Journal of Medical and Biosciences
Abbreviated Key Title: Cross Current Int J Med Biosci
ISSN: 2663-2446 (Print) & Open Access
DOI: https://doi.org/10.36344/ccijmb.2025.v07i06.006

Volume-7 | Issue-6 | Nov-Dec, 2025 |

Review Article

Preclinical Safety Assessment of the VienDO™ Venous Implantable Port: *In vitro* Cytotoxicity and *In vivo* Acute Systemic Toxicity Evaluation

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| **Received:** 26.09.2025 | **Accepted:** 21.11.2025 | **Published:** 29.11.2025 |

Abstract: The biocompatibility assessment of medical devices is a vital component of preclinical safety evaluation to ensure their compatibility with biological systems before clinical application. The present study assesses both the Cytotoxicity (*in vitro*) and acute systemic toxicity (*in vivo*) potential of the Venous Implantable Port in accordance with ISO 10993 series standards and OECD principles of Good Laboratory Practice (GLP). The *in vitro* cytotoxicity test was conducted using the L929 mouse fibroblast cell line as per ISO 10993-5:2009/EN ISO 10993-5:2009 and US FDA (FR 2-245):2016 guidelines. Cytotoxic potential was evaluated by morphological observation and the MTT colorimetric assay. The study revealed normal cell morphology and cell viability above the acceptance threshold, indicating the absence of cytotoxicity. The *in vivo* acute systemic toxicity evaluation was performed in Swiss albino mice as per ISO 10993-11:2017/EN ISO 10993-11:2018 and ISO 10993-12:2021 guidelines. Appropriate extracts of the test item were administered via different dosing routes. Throughout the observation period, no mortality, morbidity, or abnormal clinical signs were observed and gross pathological examination showed no visible tissue or organ abnormalities. Overall, the Venous Implantable Port was found to be non-cytotoxic in cytotoxicity study, and non-toxic in acute systemic toxicity study, satisfying the biological safety requirements and toxicological end points for the same as per the ISO 10993 standard.

Keywords: Animal, Biocompatibility, *In Vivo*, *In Vitro*, Polar, Non-Polar, Cell Line.

INTRODUCTION

Biocompatibility evaluation is a critical aspect of preclinical safety assessment for medical devices to ensure their compatibility with biological systems prior to clinical application. Among the battery of tests outlined in the ISO 10993 series, *in vitro* cytotoxicity serves as an essential initial screening method for detecting potential cellular toxicity of device materials. To ensure systemic toxicity potential, *in vivo* acute systemic toxicity study was also conducted. These comprehensive studies were performed to assess both the potential cytotoxicity and systemic toxicity effects thereby contributing to its overall biocompatibility profile as required under international regulatory standards for the medical device Venous Implantable Port.

According to international regulatory guidelines such as ISO 10993-5:2009/EN ISO 10993-5:2009 and US FDA (FR 2-245):2016, cytotoxicity testing assesses the biological response of cultured mammalian cells when exposed to extracts of the test material under controlled laboratory conditions. In addition to cytotoxicity evaluation, the test item underwent acute systemic toxicity assessment as per ISO 10993-11:2017/EN ISO 10993-11:2018 and US FDA (FR 2-255):2018 guidelines. The objective of this component was to evaluate potential systemic toxic effects following administration of extracts of the test item in Swiss albino mice. For both the studies, extract preparation conducted under aseptic conditions according to ISO 10993-12:2021.

Quick Response Code

Journal homepage:
https://www.easpublisher.com/

Citation: Minocha Dr. Pramodkumar, Kothwala Dr. Deveshkumar, Pandya Kamna, Ladumor Rahul, Mistry Saurabh, Kadam Aniket, Durani Ovesh, Bankeli Aakil (2025). Preclinical Safety Assessment of the VienDO™ Venous Implantable Port: *In vitro* Cytotoxicity and *In vivo* Acute Systemic Toxicity Evaluation. *Cross Current Int J Med Biosci*, *7*(6), 133-145.

Device Description

The *VienDO™ Venous Implantable Port* is intended for patient therapies requiring repeated vascular access and is suitable for the infusion of medications, intravenous fluids, parenteral nutrition, and blood products, as well as for the withdrawal of blood samples. The device assembly is composed of several materials: the venous implantable port is made from polysulfone, stainless steel 316L, silicone, and polyurethane; the guide wire is made from stainless steel 316L; the tear-away introducer sheath is composed of high-density polyethylene (HDPE); the tunneler and needle are both made from stainless steel.

The venous port has the following dimensions: the top body measures 27.65 mm in length, 24.65 mm in width, and 13.24 mm in height, while the bottom body measures 23.80 mm in length, 16.50 mm in width, and 7.70 mm in height. The bowl part assembly has diameters of 13.30 mm and 10.00 mm, a length of 28.00 mm, and a height of 10.13 mm. The silicone membrane has diameters of 9.80 mm and 11.50 mm, with a height of 5.25 mm. The catheter has an outer diameter of 3.10 mm, an inner diameter of 2.10 mm, and a total length of 700 mm. The guide wire measures 0.89 mm in diameter and 45 cm in length; the tear-away introducer sheath has a sheath outer diameter of 4.00 mm and a length of 150 mm, with the dilator measuring 3.45 mm in outer diameter and 206 mm in length. The tunneler has a diameter of 2.95 mm and a length of 225 mm, while the needle measures 1.27 mm in diameter and 111.40 mm in length. The test item has a thickness of approximately 0.5 mm, a total surface area of 28023.85 mm^2, and a net weight of 34.29634 g per unit.

Product Image

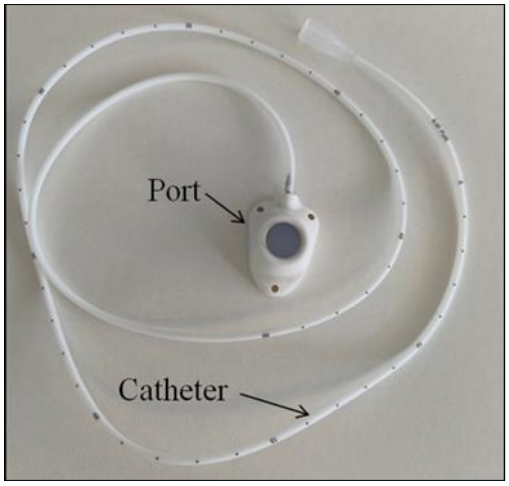


Figure 1: Actual diagram of Implantable Port with Catheter

Extraction Procedure

For cytotoxicity test, the test item having surface area 280.2385 cm^2 was placed in dry sterile container with 93.41 mL extraction media. The extraction glass container was kept in the orbital shaking incubator at 37°C and 80 rpm for 72 hrs. For acute toxicity test, the test item having surface area 280.2385 cm^2 was placed in dry sterile containers with 93.41 mL extraction media for both polar vehicle (Normal saline) and non polar vehicle (Sesame oil) separately. The extraction glass containers were kept in the orbital shaking incubator at 50°C for 72 hrs. The extraction vehicle (media) alone without test item was serve as the vehicle control. After extraction, test extracts was allowed to attain room temperature. The decanted extract was stored in sterile dry glass container at room temperature until exposure. The extracts was used without any modification (not filtered, centrifuged or pH adjusted), after device extraction.

Experimental Designe

For Cytotoxicity Test:

After completion of incubation time, the culture medium was removed from each well of the culture plate. 100 µL of MEM medium, extraction vehicle control, negative control, positive control, test item extract (100%) and diluted test extracts (50%, 25%, 12.5% and 6.25%) were added to the respective wells in triplicates. Cells were incubated further for 24 hrs with specific condition of 5% CO_2, 37°C temperature and ≥ 90 % humidity in CO_2 incubator.

After duration of exposure, each well was examined under the inverted microscope to identify systematic cell seeding errors and growth characteristics of treated cells. Microscopic examination was done to assign the reactivity grades to the morphological characteristics.

After the examination of the plates, the culture medium was removed from the well. 50 µL of the MTT solution (1 mg/mL & pH 6) was added to each test well and incubated further for 2 h in the incubator (37°C, 5 % CO2 with ≥ 90 % humidity). The MTT solution was decanted carefully and 100 µL of isopropanol was added in each well followed by incubation for 10 minutes to dissolve the formazan crystals completely. The color of

each well was observed visually. 96 well plate was transferred into a microplate reader equipped with a 570 nm filter to read the absorbance. **Safety Precaution:** Personnel working with the test item and its extracts wore personal protection such as gloves, head cap, face mask, protective body garments.

For Acute Systemic Toxicity Test:

The study was designed to use minimum number of animals to meet the scientific objectives and in consideration of requirements. For the experimental procedure, animals were divided in to the four groups having five animals each group, two groups for the vehicle controls (polar and non polar) and two groups for the test extracts (polar and non polar). Dosing routes for the animals was selected on the basis of the extraction vehicles. Intravenous and Intraperitoneal route were selected for the polar and non polar extracts respectively. All extracts were agitated vigorously prior to dosing to ensure even distribution of the extracted matter. Each of the five animals per group was treated with the test extract and vehicle control respectively according to 50 mL/kg body weight on day 1 as a single dose. Several observations were made during the course of the study.

Safety Precaution:

Personnel working with animals, test item and its extracts wore personal protection such as gloves, head cap, face mask and protective body garments.

OBSERVATIONS

For Extractions:

Following parameters were checked for extracts (Pre and post extraction) of the studies viz., appearance, colour change, presence of particulate matter and turbidity.

For Cytotoxicity:

Microscopic Examination (Qualitative Analysis)

Cells were observed for various changes such as general morphology, vacuolization, detachment, cell lysis, and membrane integrity. The change from normal morphology was recorded numerically. The reactivity of grades for cellular toxicity was designated in as mentioned in the below table 1:

Table 1: Qualitative Morphological Grading of Cytotoxicity of Extracts

Grade	Reactivity	Conditions of cell culture
0	None	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth
1	Slight	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth
2	Mild	Not more than 50 % of the cells are round, devoid of intracytoplasmatic 2 Mild granules, no extensive cell lysis; not more than 50 % growth inhibition observable
3	Moderate	Not more than 70 % of the cell layers contain rounded cells or are lysed; 3 Moderate cell layers not completely destroyed, but more than 50 % growth inhibition observable
4	Severe	Nearly complete or complete destruction of the cell layers

Note: The achievement of a numerical grade greater than 2 is considered as cytotoxic effect.

Visual Examination

The MTT was reduced to an insoluble, colored (purple) formazan product which was observed as a precipitated entity in the culture well plate. The cells were lysed with an organic solvent (e.g. Isopropanol) to solubilize the formazan which was visually observed as purple colour.

Quantitative Analysis

The spectrophotometric analysis of the culture well plate was resulted in numerical optical density at 570 nm. The average of the replicate value was calculated.

% cell viability was calculated using following equation:

% cell viability =OD570e – Od570b/ OD570c – Od570b *100

Where,

OD570e: The mean value of the measured optical density of the extracts of the test sample, negative control or positive control.

OD570c: The mean value of the measured optical density of the vehicle control.

OD570b: The mean value of the measured optical density of the blank control.

Acceptance Criteria

Table 2: Following criteria was considered for data acceptance

Criteria	Status
The mean OD_{570} of untreated control is ≥ 0.2.	Accepted
The mean of the left and right untreated controls does not differ by more than 15% from the mean of all untreated controls.	Accepted
Positive control ≥ 3 grade based on Qualitative assay.	Accepted
Vehicle control 0 grade based on Qualitative assay.	Accepted

Data Evaluation

Table 3: Following criteria were considered for data evaluation

Observation	Acceptance Criteria
The achievement of numerical grade greater than 2 based on Table 1	Cytotoxic
Purple color of formazan formation during MTT assay	Unaffected replicates
Yellowish or colorless field during MTT assay	Severely affected replicates
If cell viability is less than 70% of the vehicle control	Cytotoxic
The 50% extract of the test item showed same or a higher viability percentage than the 100% extract	Accepted

For Acute Systemic Toxicity Test:

Following observations were made during the course of the study viz., mortality/morbidity, clinical sign, body weight, clinical pathology and gross pathology to evaluate the acute systemic toxicity.

RESULTS

For Extractions:

Various parameters of the test item extracts were assessed in both the Cytotoxicity and Acute Systemic Toxicity studies. The results of these evaluations are summarized in Tables 4 and 5, respectively.

Table 4: Parameters were checked for extracts (Pre and post extraction) of Cytotoxicity test are as follows

Sr. No.	Parameters	Controls (Pre-Extraction)	Controls (Post-Extraction)	Test item without vehicle (Pre-Extraction)	Test item without vehicle (Post-Extraction)	Test item with vehicle (Pre-Extraction)	Test item with vehicle (Post-Extraction)
1	Appearance	Clear	Clear	Clear	Clear	Clear	Clear
2	Colour change	No	No	No	No	No	No
3	Presence of Particulate Matter	No	No	No	No	No	No
4	Turbidity	No	No	No	No	No	No

Table 5: Parameters were checked for extracts (Pre and post extraction) of acute systemic toxicity test are as follows

Sr. No.	Parameters	Controls (Pre-Extraction)	Controls (Post-Extraction)	Test item without vehicle (Pre-Extraction)	Test item without vehicle (Post-Extraction)
1	Appearance	Clear	Clear	Clear	Clear
2	Colour change	No	No	No	No
3	Presence of Particulate Matter	No	No	No	No
4	Turbidity	No	No	No	No

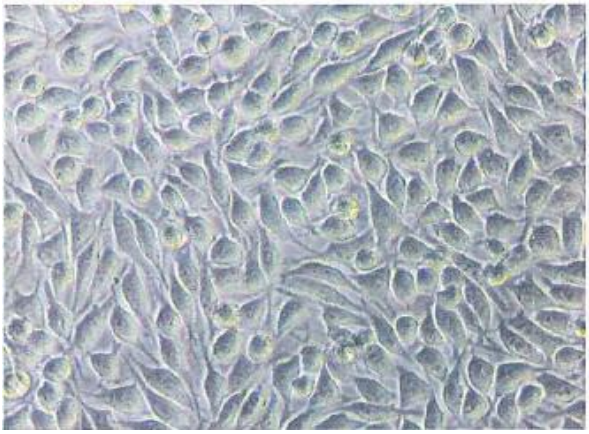
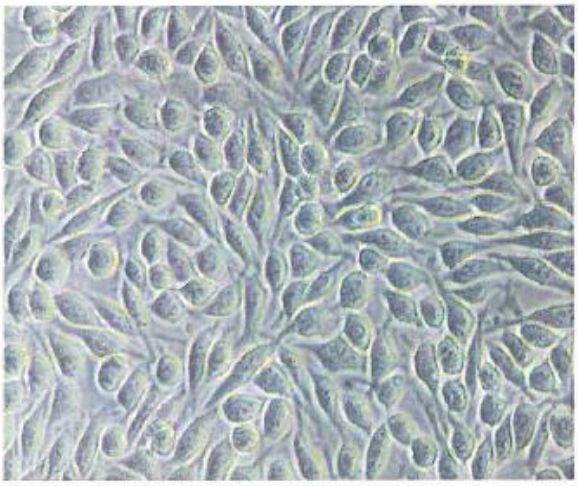
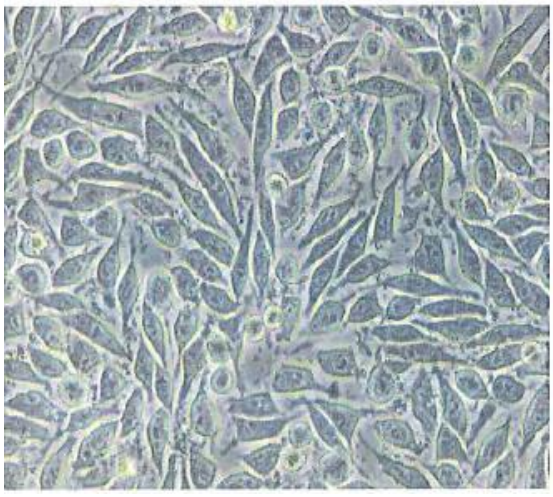
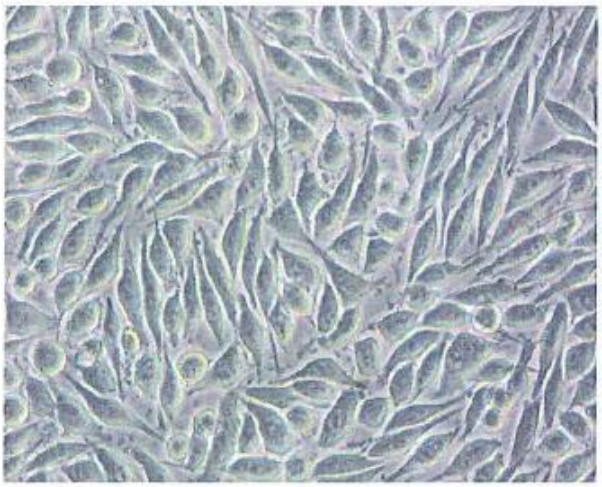
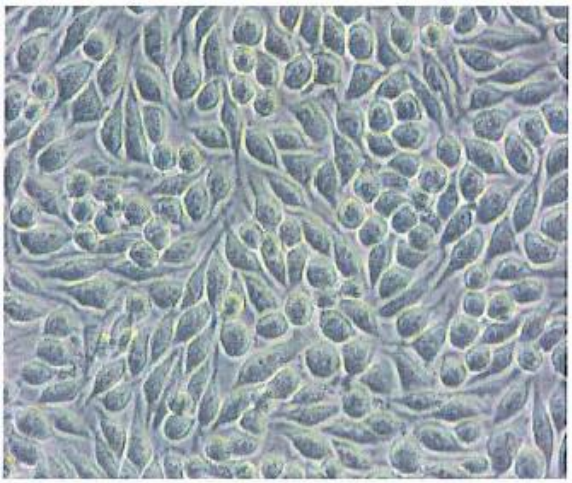
For Cytotoxicity Test:

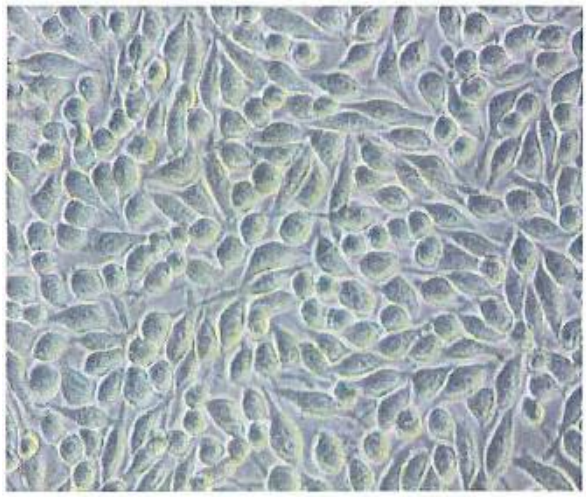
Microscopic Examination (Qualitative Analysis)

Microscopic imaging of test item concentrations and respective controls for assigning reactivity grades was done using 10X lens. Reactivity grades were assigned according to the morphological and growth characteristics as vehicle and negative control showed no reactivity whereas positive control showed severe reactivity.

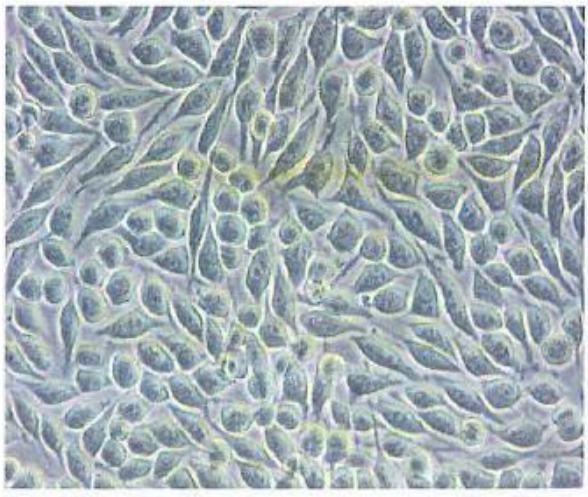
The achievement of numerical grade of "0" (reactivity: None) for undiluted extract (100%) of test item based on Table 1 was considered non-cytotoxic effect. All the diluted concentrations (50%, 25%, 12.5% and 6.25%) of test item extracts showed no reactivity and the same was mention in table 6 with microscopic examination images.

Table 6: Representative Experimental Photographs of L929 Cell Line with grading

	
Vehicle Control : Reactivity (None)	**Positive Control(ZDEC) : Reactivity (Severe) Grade:4**
	
Negative Control (HDPE), Reactivity (None) Grade: 0	**Test Item D0 : 100% , Reactivity (None) Grade: 0**
	
Test Item D1 : 50% , Reactivity (None) Grade: 0	**Test Item D2 : 25% , Reactivity (None) Grade: 0**



Test Item D3 : 12.5% , Reactivity (None) Grade: 0



Test Item D4 : 6.5% , Reactivity (None) Grade: 0

Table 7: Qualitative Morphological Grading of Cytotoxicity

Experimental Group	Numerical Reactivity Grades				Reactivity
	Replicate 1	Replicate 2	Replicate 3	Average	
Vehicle Control	0	0	0	0	None
Negative Control	0	0	0	0	None
Positive Control	4	4	4	4	Severe
Test Item Treated					
Undiluted (100%)	0	0	0	0	None
Dilution 1 (50%)	0	0	0	0	None
Dilution 2 (25%)	0	0	0	0	None
Dilution 3 (12.5%)	0	0	0	0	None
Dilution 4 (6.25%)	0	0	0	0	None

Systemic Cell Seeding Error

Untreated controls were placed on the both side of 96 well plate to check systemic cell seeding error 3 .51 % of the difference was observed between left side and right side of the plate which is Fall into acceptance criteria.

Table 8: Systematic Cell Seeding Error Check

Sample	Replicate	OD at 570 nm	Mean OD	SD for OD	Mean OD of Untreated Control	% Difference
Untreated Control (Left side)	R1	1.056	1.073	0.02	1.055	3.51
	R2	1.098				
	R3	1.065				
Untreated Control (Right side)	R1	1.027	1.036	0.02		
	R2	1.057				
	R3	1.025				

Key: R: Replicate, OD: Optical density

Quantitative Biochemical Assay

Purple colour of formazan formation was observed in the untreated controls, vehicle controls, negative controls, undiluted and the entire diluted test item extract concentrations whereas positive controls were found to be colourless during MTT assay.

92.10 % of cell viability for the undiluted extract (100%) of test item was considered as noncytotoxic. The 50% extract of the test item had higher viability percentage (94.57 %) than the 100% extract of test item.

Cell viability percentage was estimated greater than 70% for undiluted (100%) and all diluted concentrations (50%, 25%, 12.5% and 6.25%) of test item extract. Quantitative estimation of cell viability is mentioned in table 9.

Table 9: Quantitative Estimation of Cell Viability based on MTT Assay

Sample	Replicate	OD at 570 nm	Mean Blank Subtracted OD	SD for OD	% Viability (Compared to Vehicle Control)
Blank	R1	0.225	-	-	-
	R2	0.225			
	R3	0.225			
Vehicle Control (MEM Medium)	R1	1.035	1.035	0.00	100.00
	R2	1.031			
	R3	1.035			
Negative Control (HDPE)	R1	1.016	1.032	0.02	99.63
	R2	1.036			
	R3	1.032			
Positive Control (ZDEC)	R1	0.442	0.423	0.02	24.44
	R2	0.425			
	R3	0.402			
Test Item Extract					
Undiluted (100%)	R1	0.987	0.971	0.01	92.10
	R2	0.954			
	R3	0.995			
Dilution 1 (50%)	R1	0.965	0.991	0.01	94.57
	R2	0.954			
	R3	1.012			
Dilution 2 (25%)	R1	1.002	1.010	0.01	96.91
	R2	0.995			
	R3	1.013			
Dilution 3 (12.5%)	R1	1.066	1.070	0.01	97.28
	R2	1.076			
	R3	1.067			
Dilution 4 (6.25%)	R1	1.025	1.024	0.01	98.64
	R2	1.036			
	R3	1.035			

Key: OD**:** Optical density, SD: Standard deviation

For Acute Systemic Toxicity Test:

Mortality and Morbidity

No mortality or morbidity was observed in any of the animals treated with either the polar or non-polar extracts of the test item throughout the study period, indicating that the test item was well tolerated under the experimental conditions.

Table 10: Individual Animal Mortality and Morbidity Observation

Sr. No	Vehicle	Group	Animal No.	Sex	Mortality/Morbidity (Day 1–7, Morning & Evening)
1	Normal Saline	G1 (Polar Vehicle Control)	1	M	0
2	Normal Saline	G1 (Polar Vehicle Control)	2	M	0
3	Normal Saline	G1 (Polar Vehicle Control)	3	M	0
4	Normal Saline	G1 (Polar Vehicle Control)	4	M	0
5	Normal Saline	G1 (Polar Vehicle Control)	5	M	0
6	Normal Saline	G2 (Polar Test Extract)	6	M	0
7	Normal Saline	G2 (Polar Test Extract)	7	M	0
8	Normal Saline	G2 (Polar Test Extract)	8	M	0
9	Normal Saline	G2 (Polar Test Extract)	9	M	0
10	Normal Saline	G2 (Polar Test Extract)	10	M	0
11	Sesame Oil	G3 (Non-Polar Vehicle Control)	11	M	0
12	Sesame Oil	G3 (Non-Polar Vehicle Control)	12	M	0
13	Sesame Oil	G3 (Non-Polar Vehicle Control)	13	M	0
14	Sesame Oil	G3 (Non-Polar Vehicle Control)	14	M	0
15	Sesame Oil	G3 (Non-Polar Vehicle Control)	15	M	0
16	Sesame Oil	G4 (Non-Polar Test Extract)	16	M	0
17	Sesame Oil	G4 (Non-Polar Test Extract)	17	M	0

Sr. No	Vehicle	Group	Animal No.	Sex	Mortality/Morbidity (Day 1–7, Morning & Evening)
18	Sesame Oil	G4 (Non-Polar Test Extract)	18	M	0
19	Sesame Oil	G4 (Non-Polar Test Extract)	19	M	0
20	Sesame Oil	G4 (Non-Polar Test Extract)	20	M	0

Key: M: Male, 0: No Mortality/Morbidity, G: Group

Clinical Signs

All animals treated with the polar and non-polar extracts of the test item, as well as those in the respective vehicle control groups, appeared normal during the entire observation period. No signs of clinical abnormalities or behavioral changes were noted.

Table 11: Individual Animal Clinical Signs Observation

Vehicle	Group	Animal No.	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Normal Saline	G1 (Polar Vehicle Control)	1	M	1	1	1	1	1	1	1
Normal Saline	G1 (Polar Vehicle Control)	2	M	1	1	1	1	1	1	1
Normal Saline	G1 (Polar Vehicle Control)	3	M	1	1	1	1	1	1	1
Normal Saline	G1 (Polar Vehicle Control)	4	M	1	1	1	1	1	1	1
Normal Saline	G1 (Polar Vehicle Control)	5	M	1	1	1	1	1	1	1
Normal Saline	G2 (Polar Test Extract)	6	M	1	1	1	1	1	1	1
Normal Saline	G2 (Polar Test Extract)	7	M	1	1	1	1	1	1	1
Normal Saline	G2 (Polar Test Extract)	8	M	1	1	1	1	1	1	1
Normal Saline	G2 (Polar Test Extract)	9	M	1	1	1	1	1	1	1
Normal Saline	G2 (Polar Test Extract)	10	M	1	1	1	1	1	1	1
Sesame Oil	G3 (Non-Polar Vehicle Control)	11	M	1	1	1	1	1	1	1
Sesame Oil	G3 (Non-Polar Vehicle Control)	12	M	1	1	1	1	1	1	1
Sesame Oil	G3 (Non-Polar Vehicle Control)	13	M	1	1	1	1	1	1	1
Sesame Oil	G3 (Non-Polar Vehicle Control)	14	M	1	1	1	1	1	1	1
Sesame Oil	G3 (Non-Polar Vehicle Control)	15	M	1	1	1	1	1	1	1
Sesame Oil	G4 (Non-Polar Test Extract)	16	M	1	1	1	1	1	1	1
Sesame Oil	G4 (Non-Polar Test Extract)	17	M	1	1	1	1	1	1	1
Sesame Oil	G4 (Non-Polar Test Extract)	18	M	1	1	1	1	1	1	1
Sesame Oil	G4 (Non-Polar Test Extract)	19	M	1	1	1	1	1	1	1
Sesame Oil	G4 (Non-Polar Test Extract)	20	M	1	1	1	1	1	1	1

Key: M: Male, 1: Normal, G: Group

Body Weight

A gradual increase in body weight was observed in all treated and control animals over the study duration. The mean body weight on day 7 showed a consistent increase compared to the measurements recorded on days 1, 2, 3, and 4. Body weight data were expressed as mean ± standard deviation (SD) and presented in table 12 and 13. This progressive weight gain indicates the absence of any adverse systemic effect on the animals' normal growth.

Table 12: Individual Animal Body Weight and Group Mean Values

Vehicle	Group	Animal No.	Sex	Body Weight (g)				
				Day 1	Day 2	Day 3	Day 4	Day 7
Normal Saline	G1 (Polar Vehicle Control)	1	M	22.43	23.27	22.7	22.83	23.36
Normal Saline	G1 (Polar Vehicle Control)	2	M	25.82	25.97	26.22	26.26	26.83
Normal Saline	G1 (Polar Vehicle Control)	3	M	26.18	26.93	26.91	27.43	28.13
Normal Saline	G1 (Polar Vehicle Control)	4	M	27.16	27.33	27.47	27.62	28.23
Normal Saline	G1 (Polar Vehicle Control)	5	M	27.75	27.97	28.07	28.18	28.55
	Mean			25.868	26.294	26.274	26.464	27.02
	SD			2.069461283	1.839587	2.111689	2.148355	2.148767
	No. Of animal			5	5	5	5	5
Normal Saline	G2 (Polar Test Extract)	6	M	23.7	24.53	24.81	25.03	25.33
Normal Saline	G2 (Polar Test Extract)	7	M	24.56	24.9	25.33	25.31	25.81
Normal Saline	G2 (Polar Test Extract)	8	M	25.41	25.73	25.95	26.01	26.55
Normal Saline	G2 (Polar Test Extract)	9	M	26.48	26.71	27	27.13	27.51
Normal Saline	G2 (Polar Test Extract)	10	M	27.51	27.96	28.06	28.11	28.81
	Mean			25.532	25.966	26.23	26.318	26.802
	SD			1.510552879	1.394966	1.3083	1.288456	1.391733
	No. Of animal			5	5	5	5	5
Sesame Oil	G3 (Non-Polar Vehicle Control)	11	M	23.35	23.5	23.66	23.91	24.31
Sesame Oil	G3 (Non-Polar Vehicle Control)	12	M	24.34	24.94	25.01	25.21	25.83
Sesame Oil	G3 (Non-Polar Vehicle Control)	13	M	25.45	25.83	26.17	26.37	26.73
Sesame Oil	G3 (Non-Polar Vehicle Control)	14	M	26.15	26.74	27.03	27.36	27.91
Sesame Oil	G3 (Non-Polar Vehicle Control)	15	M	27.06	27.54	27.83	28.11	28.71
	Mean			25.27	25.71	25.94	26.192	26.698
	SD			1.463061858	1.573309	1.648969	1.676789	1.730179
	No. Of animal			5	5	5	5	5
Sesame Oil	G4 (Non-Polar Test Extract)	16	M	23.88	24.03	24.2	24.36	24.8
Sesame Oil	G4 (Non-Polar Test Extract)	17	M	25.55	25.68	25.81	25.94	26.45
Sesame Oil	G4 (Non-Polar Test Extract)	18	M	26.17	26.31	26.46	26.61	27.07
Sesame Oil	G4 (Non-Polar Test Extract)	19	M	26.89	27.05	27.21	27.37	27.82

Sesame Oil	G4 (Non-Polar Test Extract)	20	M	29.53	29.68	29.82	29.96	30.4
	Mean			26.404	26.55	26.7	26.848	27.308
	SD			2.071395665	2.074355	2.067257	2.063267	2.055814
	No. of Animal			5	5	5	5	5

Key: M: Male, SD: Standard Deviation, G: Group

Table 13: Individual Animal Body Weight Change and Group Mean Values

Vehicle	**Group**	**Animal No.**	**Sex**	**Day 2**	**Day 3**	**Day 4**	**Day 7**
Normal Saline	G1(Polar Vehicle Control)	1	M	0.62	1.2	1.37	4.15
Normal Saline	G1(Polar Vehicle Control)	2	M	0.59	1.05	1.64	3.6
Normal Saline	G1 (Polar Vehicle Control)	3	M	0.57	1.15	1.63	3.4
Normal Saline	G1 (Polar Vehicle Control)	4	M	0.49	1.04	1.52	3.25
Normal Saline	G1 (Polar Vehicle Control)	5	M	0.47	1.05	1.62	3.51
	Mean			0.548	1.098	1.556	3.582
	SD			0.064962	0.072595	0.114586	0.343322
	No. Of animal			5	5	5	5
Normal Saline	G2 (Polar Test Extract)	6	M	0.63	1.23	1.39	3.89
Normal Saline	G2 (Polar Test Extract)	7	M	0.61	1.2	1.35	3.67
Normal Saline	G2 (Polar Test Extract)	8	M	0.58	1.17	1.31	3.53
Normal Saline	G2 (Polar Test Extract)	9	M	0.57	1.14	1.29	3.38
Normal Saline	G2 (Polar Test Extract)	10	M	0.55	1.13	1.27	3.27
	Mean			0.588	1.174	1.322	3.548
	SD			0.031937	0.041593	0.048166	0.243762
	No. Of animal			5	5	5	5
Sesame Oil	G3 (Non-Polar Vehicle Control)	11	M	0.64	1.33	1.57	4.28
Sesame Oil	G3 (Non-Polar Vehicle Control)	12	M	0.61	1.3	1.52	4.05
Sesame Oil	G3 (Non-Polar Vehicle Control)	13	M	0.59	1.25	1.48	3.83
Sesame Oil	G3 (Non-Polar Vehicle Control)	14	M	0.57	1.22	1.43	3.62
Sesame Oil	G3 (Non-Polar Vehicle Control)	15	M	0.55	1.18	1.39	3.45
	Mean			0.592	1.256	1.478	3.846
	SD			0.034928	0.060249	0.071204	0.330953
	No. Of animal			5	5	5	5
Sesame Oil	G4 (Non-Polar Test Extract)	16	M	0.63	1.34	2.01	3.85
Sesame Oil	G4 (Non-Polar Test Extract)	17	M	0.51	1.02	1.53	3.52
Sesame Oil	G4 (Non-Polar Test Extract)	18	M	0.53	1.11	1.68	3.44
Sesame Oil	G4 (Non-Polar Test Extract)	19	M	0.6	1.19	1.79	3.46
Sesame Oil	G4 (Non-Polar Test Extract)	20	M	0.91	0.98	1.46	3.06
	Mean			0.636	1.128	1.694	3.466
	SD			0.160873	0.143771	0.218472	0.281034
	No. Of animal			5	5	5	5

Key: M: Male, SD: Standard Deviation, G: Group

Gross Pathology

Gross pathological examination of all treated animals revealed no visible abnormalities or lesions. All organs appeared normal in size, color, and texture, indicating no treatment-related pathological changes (Table 14).

Table 14: Individual Animal Gross Pathology

Vehicle	Group	Animal No.	Sex	Reason of Euthanasia	Gross (Macroscopic) Observations
Normal Saline	G1 (Polar Vehicle Control)	1	M	Terminal	No Abnormality Detected
Normal Saline	G1 (Polar Vehicle Control)	2	M	Terminal	No Abnormality Detected
Normal Saline	G1 (Polar Vehicle Control)	3	M	Terminal	No Abnormality Detected
Normal Saline	G1 (Polar Vehicle Control)	4	M	Terminal	No Abnormality Detected
Normal Saline	G1 (Polar Vehicle Control)	5	M	Terminal	No Abnormality Detected
Normal Saline	G2 (Polar Test Extract)	6	M	Terminal	No Abnormality Detected
Normal Saline	G2 (Polar Test Extract)	7	M	Terminal	No Abnormality Detected
Normal Saline	G2 (Polar Test Extract)	8	M	Terminal	No Abnormality Detected
Normal Saline	G2 (Polar Test Extract)	9	M	Terminal	No Abnormality Detected
Normal Saline	G2 (Polar Test Extract)	10	M	Terminal	No Abnormality Detected
Sesame Oil	G3 (Non-Polar Vehicle Control)	11	M	Terminal	No Abnormality Detected
Sesame Oil	G3 (Non-Polar Vehicle Control)	12	M	Terminal	No Abnormality Detected
Sesame Oil	G3 (Non-Polar Vehicle Control)	13	M	Terminal	No Abnormality Detected
Sesame Oil	G3 (Non-Polar Vehicle Control)	14	M	Terminal	No Abnormality Detected
Sesame Oil	G3 (Non-Polar Vehicle Control)	15	M	Terminal	No Abnormality Detected
Sesame Oil	G4 (Non-Polar Test Extract)	16	M	Terminal	No Abnormality Detected
Sesame Oil	G4 (Non-Polar Test Extract)	17	M	Terminal	No Abnormality Detected
Sesame Oil	G4 (Non-Polar Test Extract)	18	M	Terminal	No Abnormality Detected
Sesame Oil	G4 (Non-Polar Test Extract)	19	M	Terminal	No Abnormality Detected
Sesame Oil	G4 (Non-Polar Test Extract)	20	M	Terminal	No Abnormality Detected

Key: M: Male, SD: Standard Deviation, G: Group

Statistical Analysis

Statistical analysis of the body weight data was performed using one-way ANOVA with a 95% confidence interval, employing GraphPad Prism Software (version 9.3.1). The analysis showed no statistically significant differences in body weight among the treatment and control groups, confirming the absence of treatment-related effects.

DISSCUSION

For Cytotoxicity Test:

The cytotoxic potential of the device was evaluated using the L929 mouse fibroblast cell line in accordance with ISO 10993-5:2009/EN ISO 10993-5:2009 and US FDA (FR 2-245):2016 guidelines and principles of Good Laboratory Practice (GLP). The studies assess the biocompatibility of the test material by determining its influence on cell morphology and metabolic activity through the MTT assay. The microscopic examination revealed normal cellular morphology in the untreated, vehicle control, negative control, undiluted, and all diluted extract concentrations of the test item. The presence of intense purple coloration of formazan crystals in these groups confirmed the metabolic activity of viable cells, thereby indicating the absence of cytotoxic effects. In contrast, the positive control exhibited a colorless field, confirming the validity and sensitivity of the assay.

The quantitative assessment of cell viability using the MTT assay demonstrated that the undiluted (100%) extract of the test item yielded a cell viability of 92.10%, which is well above the cytotoxicity threshold of 70% as stipulated in ISO 10993-5. Consequently, the test material was classified under reactivity grade 0 (None), signifying the absence of cytotoxic potential. Similarly, all diluted concentrations of the test extract (50%, 25%, 12.5%, and 6.25%) exhibited viability percentages exceeding the acceptance limit, further supporting the non-cytotoxic nature of the material of the device.

For Acute Systemic Toxicity Test:

The potential acute systemic toxicity of the test item evaluated by administration of its polar and non-polar extracts, in compliance with ISO 10993-11: 201 7 / EN ISO I 0993-11: 20 18 / US FDA (FR 2-255):2018 and principles of Good Laboratory Practice (GLP).

Throughout the observation period, no mortality or morbidity was recorded in any of the test or control groups, suggesting the absence of acute toxic manifestations. Clinical examinations of animals were performed at regular intervals revealed no abnormal behavioral patterns, neurological symptoms, or signs of systemic distress in animals administered either extract of the test item. The general health and activity of the animals remained comparable to that of the vehicle control group, thereby confirming that the test material was physiologically well-tolerated.

Body weight progression, a sensitive parameter for assessing the overall systemic impact of xenobiotic substances, showed a consistent increase across all groups. Statistical analysis using one-way analysis of variance (ANOVA) at a 95% confidence interval (GraphPad Prism, Version 9.3.1) revealed no significant intergroup variations in mean body weights. These

findings indicate that administration of the test item extracts did not interfere with normal metabolic processes or growth patterns.

Gross pathological examinations conducted at the termination of the study revealed no macroscopic lesions, discolorations, or tissue abnormalities in any of the major organs examined. The absence of gross pathological alterations further substantiates the non-toxic nature of the test item and confirms that neither the polar nor non-polar extract induced any observable tissue damage or organ-specific toxicity.

CONCLUSION

During Cytotoxicity test, the consistent viability across all tested concentrations, coupled with the absence of morphological alterations such as cell rounding, granulation, or lysis, strongly suggests that the test item does not release leachable substances capable of inducing cellular damage or metabolic inhibition. These findings collectively demonstrate that the test material is biocompatible and non-cytotoxic under *in vitro* conditions.

Furthermore during the acute systemic toxicity test, the absence of mortality, morbidity, clinical abnormalities, statistically significant changes in body weight, or pathological findings provides compelling evidence that the test item exhibits no acute systemic toxicity. The results affirm that the material is biocompatible and physiologically inert. The study findings conclusively demonstrated that the test material did not elicit any adverse systemic response in the treated animals under the experimental conditions employed.

Based on the findings of the present investigation collectively, results provide substantial evidence that the VienDO™ Venous Implantable Port is biocompatible, non-cytotoxic, and systemically safe when evaluated through both *in vitro* and *in vivo* biocompatibility assessments.

The material satisfies the biological safety criteria established under the applicable testing standards, confirming its appropriateness for use as a medical device.

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