PRADO
advance the safety evaluation

Preclinical Research and Development Organization

Study Report

Study Number: PRADO/TOX-260

Study Title

Acute Toxicity Study of VEDICINALS™ by Oral Route in Sprague Dawley Rats

June, 2020

Study Director

Subhashis Paul, M. Sc.

TEST FACILITY

PRADO
Preclinical Research and Development Organization, Pvt. Ltd., Survey No. 170/1, Punawale Road, Tathawade,
Pune 411 033 INDIA
Phone: +91-7770040105

SPONSOR/ SPONSOR'S REPRESENTATIVE

Mr. Prakash Salunke
VEDICINALS INDIA PRIVATE LIMITED
(Formerly known as IGES efficiency solutions India Private Limited)
J-204, Devi Indrayani Apartments,
Dehu - Alandi Road, Talawade,
Pune, 411062
E Mail: prakash160876@gmail.com
Phone: 7567499944

Confidential

CONTENTS

GLP COMPLIANCE STATEMENT

Study Title: Acute Toxicity Study of VEDICINALS^{TM} by Oral Route in Sprague Dawley Rats.

This study was conducted in the Test Facility of PRADO-Preclinical Research and Development Organization, Private Limited. I accept responsibility for the conduct of the study and validity of data and hereby declare that the study was performed under my direction and the report is complete, true and accurate representation of study raw data. There were no circumstances that might have affected the quality or integrity of the study.

This study was conducted in compliance with OECD principles of GLP (as revised in 1997, issued January 1998, ENV/MC/CHEM (98) 17) and in accordance with the written study plan, authorized by the Test Facility Management and following Standard Operating Procedures (SOPs) at PRADO.

For Test Facility

Study Director:

(Subhashis Paul)	(----------)	Jan 29, 2020 (----------)
(Name)	(Sign)	(Date)

STATEMENT OF QUALITY ASSUARANCE

Study Title: Acute Toxicity Study of VEDICINALS™ by Oral Route in Sprague Dawley Rats. The study was inspected at different phases by the Quality Assurance Unit of PRADO-Preclinical Research and Development Organization, Private limited. The final report was inspected with respect to the study plan, standard operating procedures to be followed and the raw data generated in this study. I certify that the final report is a true reflection of the raw data.

The date on which the observations were reported to the Test Facility Management and the Study Director are given below:

Serial No.	Phase	Date of Inspection / Audit	Date of Reporting to the	
			Study Director	Test Facility Management
1	Draft study plan	May 25,2020	May 25,2020	May 25,2020
2	Dose administrations	May 26,2020	May 26,2020	May 26,2020
3	Necropsy and Gross pathology	Jun 15,2020	Jun 15,2020	Jun 15,2020
4	Draft report and raw data	Jun 21,2020	Jun 21,2020	Jun 21,2020

Head, Quality Assurance Unit:

(Poornima Shinde) (Name) (----------) (Sign) Jun 29, 2020 (----------) (Date)

PERSONNEL INVOLVED IN THE STUDY

Study Director	:	Subhashis Paul, M.Sc.
Deputy Study Director	:	Anjali Lewate, M.Sc.
Study Personnel	:	Amol Kamble, B.Sc.
	:	Shweta Waghmare, B.Sc. (pursuing)
Study Veterinarian	:	Krishna Bohrey, M.V.Sc.
Study Pathologist		Sneha Thorat, M.V.Sc., Ph.D.
Quality Assurance Unit	:	Sachin Shinde, Ph.D., PGDRA, MDRA
	:	Poornima Shinde, B.Pharm

REPORT APPROVAL

This Study Report for Study number PRADO/TOX-260 'Acute Toxicity Study of VEDICINALS™ by Oral Route in Sprague Dawley Rats' has been mutually agreed and approved between:

For Test Facility

Study Director:

(Subhashis Paul)	(----------)	Jun 29, 2020
(Name)	(Sign)	(Date)

Test Facility Management:

(Dr. Pralhad Wangikar)	(----------)	Jun 29, 2020
(Name)	(Sign)	(Date)

LIST OF ABBREVIATIONS

^0C	Degree Celsius
%	Percentage
µL	Micro liter
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
Gm	Gram
Hrs	Hours
IAEC	Institutional Animal Ethics Committee
Kg	Kilogram
Ltd	Limited
LD50	Median Lethal Dose
MTD	Maximum Tolerated Dose
Mg	Milligram
Ml	Milliliter
N	Number of Animals
NAD	No Abnormalities Detected
Pvt	Private
SD	Standard Deviation
SOP	Standard Operating Procedures

SUMMARY

Study No.	PRADO/TOX-260
Test Item	VEDICINALS™
Study	Acute Toxicity Study in Rats
Route	Oral
Dose	2000 mg/kg
No. of Groups	2 (3 Females/Group)

The objective of the study was to determine the possible health hazards likely to arise in Sprague Dawley healthy Rats following single oral exposure of VEDICINALS™ followed by a 14-day observation period. The study was estimated to provide LD_{50} cut-off value and LD_{50} range as per GHS. VEDICINALS™ was supplied by Vedicinals India Private Limited (Formerly known as IGES efficiency solutions India Private Limited), Pune, India. The study was performed in accordance with OECD guideline for testing of chemicals, No. 423, entitled 'Acute Oral Toxicity- Acute toxic class method'.

The test item was initially tested at a dose of 2000 mg /kg of body weight in group G1 with 3 female rats. There was no mortality at this tested dose level hence another set of 3 female rats (Group G2) dosed with same dose, i. e. 2000 mg /kg of body weight. There was no mortality at this tested dose level. This confirmed end of the study without the need of further testing.

All the animals survived till the scheduled necropsy. No any abnormal clinical signs were observed in any animal throughout experiment period.

There was an increase in body weight of all the animals of G1 and G2 on day 8 and day 15 compared to day 1.

Gross-pathological examination of all animals of G1 and G2 did not revealed any pathological changes except animal No. 26005 (minimal multiple necrotic foci on liver). This was not considered to be related to the treatment of test item.

Based on the results of this study, i.e. 'Acute Toxicity Study of VEDICINALS™ by Oral Route in Sprague Dawley Rats', the Median Lethal Dose (LD_{50}) of VEDICINALS™ upon a single oral administration to female Sprague Dawley rats, in accordance with Globally Harmonized Classification System is **Category 5 (>5000 mg/kg of body weight)**.

The LD_{50} cut off value is 5000 mg/kg of body weight.

Globally Harmonized Classification and Labelling of Chemicals: Category 5.

1.0 INTRODUCTION

1.1 Objective

The objective of the study was to determine the possible health hazards likely to arise in Sprague Dawley healthy Rats following single oral exposure of VEDICINALS^{TM} followed by a 14-day observation period. The study was estimated to provide LD_{50} cut-off value and LD_{50} range as per GHS.

1.2 Study Guidelines

The design and scope of the study is based on consideration of the study objectives. The experimental procedures were performed on the basis of standards set forth in

OECD guideline for testing of chemicals, No. 423, entitled 'Acute Oral Toxicity- Acute toxic class method'. The Organization for Economic co-operation and Development (OECD) Guidelines for the Testing of chemicals, adopted by the council on 17 December 2001.

1.3 Good Laboratory Practice

This study was performed following the OECD Principles on Good Laboratory Practice (as revised in 1997, issued January 1998, ENV/MC/CHEM (98) 17).

Certification & Accreditation: The test facility is certified (GLP/C-127/2018) by the National GLP Compliance Monitoring Authority (NGCMA), Department of Science & Technology, Govt. of India, for compliance to OECD-GLP and by CPCSEA (1723/PO/RcBiBt/S/13/ CPCSEA) for conducting experiments on small laboratory animals.

1.4 Quality Assurance Unit

The QAU has review the draft Study Plan, inspect/audit various phases of the study conduct, inspected raw data and draft report.

1.5 Study Period

Study Initiation Date	:	May 25, 2020
Experiment Start Date	:	May 25, 2020
Experiment Completion Date	:	Jun 15, 2020
Study Completion Date	:	Jun 29, 2020

2.0 MATERIALS AND METHODS

Details of the methods mentioned in the subsequent section of the study plan are as per the appropriate Standard Operating Procedures (SOPs) of PRADO.

2.1 Test Item Details

The details of test item were given below as per TIIS and CoA (Annexure - I) provided by sponsor. The integrity of supplied data relating to the test item is the responsibility of sponsor

Name of Test Item	:	VEDICINALS™
Batch Number	:	TRL/001
Assay	:	Each 5279 mg contains - Compound 1 (85%) : 353 mg - Plant extract 1 Compound 2 + 3 (95%) : 736 mg - Plant extract 2 Compound 4 (20%) : 1500 mg - Plant extract 3 Compound 5 (90%) : 667 mg - Plant extract 4 Compound 6 (95%) : 1053 mg - Plant extract 5 Compound 7 (50%) : 889 mg - Plant extract 6 Compound 8 (95%) : 21 mg - Plant extract 7 Compound 9 (20%) : 60 mg - Plant extract 8
Manufacturing Date	:	24/05/2020
Retest Date	:	23/05/2021
Description	:	Brownish Yellow powder
Storage Conditions	:	Room temperature (20° - 30° C)

Note: Refer CoA for batch number, Assay, Manufacturing Date and Description. Expiry date and Storage condition is as per TIIS.

Note: The test item has three synonyms (CORONASH ™ / VEDICINALS ™ - 9 / MOLECUSAN ™ - 9) but VEDICINALS ™ is considered as primary name of the test iten throughout the study

2.2 Vehicle Details

Name of Vehicle	:	0.5 % Carboxy Methyl Cellulose in deionized water.
Batch Number	:	0000374509
Manufacturing Date	:	Jan 2019
Expiry / Retest Date	:	Jan 2023
Storage Conditions	:	Room Temperature
Appearance	:	Clear solution

2.3 Test System Details

Species (Strain)	:	Rat (Sprague Dawley)
Sex	:	Female. Female were nulliparous and non-pregnant.
Age (at initiation of dosing)	:	8-12 weeks
Body weight (at initiation of dosing)	:	204.0 – 211.5 g
Source	:	PRADO Pvt. Ltd., Pune (1723/PO/ReBiBt/S/13/CPCSEA)

2.4 Justification for Selection of Test System

Rat is one of the test system used for toxicity testing and accepted globally by many regulatory authorities and is also as per sponsor's requirement.

3.0 EXPERIMENTAL PROCEDURES

3.1 Animal Welfare

All procedures to be followed for the conduct of this study were in accordance with the relevant Standard Operating Procedures followed at PRADO, Pune and the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) as published in The Gazette of India, December 15, 1998. Prior approval of the Institutional Animal Ethics Committee (IAEC) was in place (IAEC-20-011).

3.2 Husbandry

Location	:	ARF Room No. 04
Temperature	:	21.2 to 23.7 °C
Humidity	:	42 to 63 %
Lighting	:	Photoperiod was 12 hrs light and 12 hrs dark. Light hours being controlled by an automated system.
Air Changes	:	10 to 15 air changes per hour were maintained throughout the in-life phase of this study.
Cages	:	3 animals per sex per cage were housed together in the polycarbonate cages.
Cage Dimensions	:	41.0 cm x 28.2 cm x 15.2 cm
Feed	:	Standard rodent diet was provided *ad libitum*.
Water	:	Reverse Osmosis water treated with ultraviolet light was provided *ad libitum* in autoclaved polypropylene bottles.
Analysis of feed, water	:	Contaminant and nutrient content analysis of feed and pesticide analysis of water samples were done routinely. Details have been kept in raw data file.
Animal Identification	:	Animals were identified by tail marking throughout the study period. Group of animals per cage were identified by different colour cage card.

3.3 Acclimatization

Total 6 Sprague Dawley Rats (Female) were issued by Animal Research Facility for this study and were allowed to acclimatize for a period of 05 days for G1 group and 07 days for G2 group.

3.4 Randomization and Grouping

After completion of acclimatization period, the animals were manually selected and grouped in such a way that the weight variation were within ± 20% of means of all dosed animals. To enable this, the animals with highest body weight were selected at each step and grouped.

3.5 Preparation of the Dose Formulation

For each dose, required quantity of the Test Item was weighed on butter paper/and transferred to the mortar and pestle. Test Item was triturated and small amount of vehicle was added in sequential manner while trituration. Then this prepared Test Item formulation was transferred to the measuring cylinder. Small amount of vehicle was added to mortar to recover traces of Test Item. This procedure of rinsing was repeated for 2-3 times. The final volume was made up to required amount with vehicle. The Test Item formulation was transferred to a suitable labelled container. The exact quantity of Test Item and vehicle used were recorded in the raw data.

3.6 Experimental Design

Groups	Doses (mg /kg of body weight)	Test Item (mg) *	Concentrations (mg/mL)	Animal Numbers
				Female
G1, Treatment	2000	2829	200	26001 -26003
G2, Treatment	2000	2829	200	26004 -26006

* Correction factor 1.4 was added for the dose calculation as per sponsor requirement.

3.7 Justification for Selection of Dose and Route of Administration

VEDICINALS™ was administered orally at doses of 2000 mg/kg of body weight for G1group. As there was no mortality observed in the first dose (2000 mg/kg of body weight), further G2 group animals were dosed with same dose (2000 mg/kg of body weight) as per OECD TG 423 Annexure 2d. Absence of test item related mortality at both the dose groups determine no further testing is needed. Dosage of VEDICINALS™ selected for this study is based on the guideline and also in line with Sponsor's requirement.

The oral route of administration is selected as per the sponsor's request as it is the intended clinical route of administration.

3.8 Dose Administration

All the animals were fasted overnight prior to dose administration. The test item was administered using stainless steel oral gavage needle (18 gauge) to each animal as a single dose. The dose volume administered to individual animal was adjusted according to body weight that

was recorded just before dosing to give a constant dosage volume of 10 ml/kg body weight. All the animals were provided with food two hours post Test Item administration.

4.0 OBSERVATIONS

Following observations were recorded from all the animals.

4.1 Mortality and Clinical Observations

After dose administration, all the animals were observed individually for clinical signs, once during the first 30 minutes, 1, 2 and 4 hours and once daily thereafter, for 14 days. All the animals were observed for mortality and morbidity least twice daily on all the days except on the day of sacrifice where it was done once. The time course of toxic effects and the reversibility were recorded.

4.2 Body Weight

Body weight was recorded on before selection, before dosing (fasting) and weekly thereafter.

4.3 Necropsy and Gross Pathology

At termination, all animals were humanely euthanized. All animals were subjected to detailed gross pathological examination which included careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

4.4 Data Analysis and Report Preparation

All the individual animal data for clinical signs, body weight, gross pathology were tabulated. Mean and Standard Deviation of body weight for each treatment group were calculated and presented in summary tables.

4.5 Archives

All original raw data, QAU reviewed draft Study Plan and draft report, approved copies of final Study Plan and final report along with electronic data files (DVD) as PDF copies of final Study Plan and Report of the study will be retained for 9 years from the date of study completion or from the date of archival at PRADO Pvt. Ltd., Thereafter, the archived material will be disposed of or stored for an extended period according to the written instructions of the Sponsor.

5.0 RESULTS AND DISCUSSION

5.1 Mortality and Clinical Observations

All the animals survived till the scheduled necropsy. No any abnormal clinical signs were observed in any animal throughout experiment period. **(Table 1, Appendix I)**

5.2 Body Weight

There was an increase in body weight of all the animals of G1 and G2 on day 8 and day 15 compared to day 1. **(Table 2 and 3, Appendix II and III)**

5.3 Necropsy and Gross Pathology

Gross-pathological examination of all animals of G1 and G2 did not revealed any pathological changes except animal No. 26005 (minimal multiple necrotic foci on liver). This was not considered to be related to the treatment of test item. **(Table 4, Appendix IV)**

6.0 CONCLUSION

Based on the results of this study, i.e. 'Acute Toxicity Study of VEDICINALS™ by Oral Route in Sprague Dawley Rats', the Median Lethal Dose (LD_{50}) of VEDICINALS™ upon a single oral administration to female Sprague Dawley rats, in accordance with Globally Harmonized Classification System is **Category 5 (>5000 mg/kg of body weight)**.

The LD_{50} cut off value is 5000 mg/kg of body weight.

Globally Harmonized Classification and Labelling of Chemicals: Category 5.

7.0 STUDY PLAN AMENDMENT AND DEVIATIONS

There was no amendment and deviation to the study plan.

8.0 REFERENCES

- CPCSEA Guidelines for laboratory animal facility, Indian Journal of Pharmacology 2015; 35: 257-274.
- OECD Principles on Good Laboratory Practice (revised 1997, issued January 1998) ENV/MC/CHEM (98) 17 Environment Directorate, Organization for Economic Co-operation and Development, Paris, 1998.
- OECD guideline for testing of chemicals, No. 423, entitled 'Acute Oral Toxicity- Acute toxic class method. The Organization for Economic co-operation and Development (OECD) Guidelines for the Testing of chemicals, adopted by the council on 17 December 200.

SUMMARY TABLE 1: Morbidity and Mortality

Sex: Female

Groups	G1	G2
Dose (mg/kg)	2000	2000
No. of Animals	3	3
Mortality	0	0
Normal	3	3

SUMMARY TABLE 2: Body Weight (gm)

Sex: Female

Mean/SD/N	Days		
	1	8	15
G1			**Dose: 2000 mg/kg**
Mean	208.67	219.17	229.83
SD	2.57	3.33	6.25
N	3	3	3
G2			**Dose: 2000 mg/kg**
Mean	206.50	219.00	235.50
SD	2.78	2.29	4.44
N	3	3	3

SUMMARY TABLE 3: Body Weight Gain (gm)

Sex: Female

Mean/SD/N	Days	
	8	15
G1		**Dose: 2000 mg/kg**
Mean	10.50	21.17
SD	1.00	4.04
N	3	3
G2		**Dose: 2000 mg/kg**
Mean	12.50	29.00
SD	1.80	2.29
N	3	3

SUMMARY TABLE 4: Gross Pathology Observations

Sex: Female

Group	G1	G2
Dose (mg/kg)	**2000**	**2000**
Number Examined	3	3
Liver- Minimal multiple necrotic foci	0	1
NAD	3	2

Keys: NAD - No Abnormality Detected

APPENDIX I: Individual Animal Clinical Signs

Sex: Female

Animal No.	Observation Days																	
	1				2	3	4	5	6	7	8	9	10	11	12	13	14	15
	30 min	1 hr	2 hr	4 hr														
G1																	**Dose: 2000 mg/kg**	
26001	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
26002	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
26003	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
G2																	**Dose: 2000 mg/kg**	
26004	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
26005	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
26006	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Keys: N - Normal

APPENDIX II: Individual Animal Body Weight (gm)

Sex: Female

Animal No.	Days		
	1	8	15
G1			**Dose: 2000 mg/kg**
26001	211.5	223.0	237.0
26002	208.0	217.5	225.5
26003	206.5	217.0	227.0
G2			**Dose: 2000 mg/kg**
26004	209.5	221.5	239.0
26005	206.0	217.0	237.0
26006	204.0	218.5	230.5

APPENDIX II: Individual Animal Body Weight Gain (gm)

Sex: Female

Animal No.	Days	
	8	15
G1		**Dose: 2000 mg/kg**
26001	11.5	25.5
26002	9.5	17.5
26003	10.5	20.5
G2		**Dose: 2000 mg/kg**
26004	12.0	29.5
26005	11.0	31.0
26006	14.5	26.5

APPENDIX IV: Individual Animal Gross Pathology

Sex: Female

Animal No.	Organ	Observations	
		External	Internal
G1			**Dose: 2000 mg/kg**
26001	-	NAD	NAD
26002	-	NAD	NAD
26003	-	NAD	NAD
G2			**Dose: 2000 mg/kg**
26004	-	NAD	NAD
26005	Liver	NAD	Minimal multiple necrotic foci
26006	-	NAD	NAD

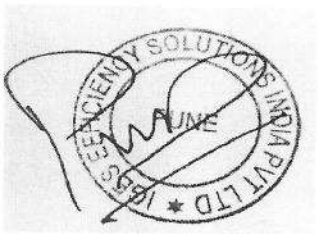
ANNEXURE I: CERTIFICATE OF ANALYSIS OF TEST ITEM

VEDICINALS INDIA PRIVATE LIMITED

REGD. OFF: Building J, Flat No. 204, Devi Indrayani Apartments, Dehu-Alandi Road, Talawade Pune Maharashtra 412114

CIN: U74999PN2017PTC170824 : Email: prakash160876@gmail.com

Certificate of Analysis / Specifications		
Sample Name: CORONASH ™ / VEDICINALS ™ - 9 / MOLECUSAN ™ - 9	**Project ID: CORONASH ™ / VEDICINALS ™ - 9 / MOLECUSAN ™ - 9**	**Batch No. : TRL/001**
Mfg Date: 24/05/2020	**Shelf life: 2 Years**	**Batch Qty: 500 gm**
Analysis / Specifications		
Description	Brownish Yellow powder	
Solubility	Slightly soluble in Water, Soluble in Ethyl Alcohol and DMSO	
Average Weight	5279 mg ± 2.5%	
Weight Variation	5279 mg ± 5%	
Residual Solvents	Complies IP/USP	
LOD	NMT 10%	
Strength/Potency/Assay of formulation.	**Each 5279 mg contains -** Compound 1 (85%) : 353 mg – Plant extract 1 Compound 2 + 3 (95%) : 736 mg - Plant extract 2 Compound 4 (20%) : 1500 mg - Plant extract 3 Compound 5 (90%) : 667 mg - Plant extract 4 Compound 6 (95%) : 1053 mg – Plant extract 5 Compound 7 (50%) : 889 mg – Plant extract 6 Compound 8 (95%) : 21 mg - Plant extract 7 Compound 9 (20%) : 60 mg - Plant extract 8 Individual COA of each Phyto-compound with manufacturing batch details available.	
Mocrobological Testing	All materials used in the preparation of CORONASH ™ / VEDICINALS ™ - 9/ MOLECUSAN ™ – 9 powder complies as per requirements of IP/USP	



For VEDICINALS INDIA PVT LTD
DIRECTOR

Note: The test item has three synonyms (CORONASH ™ / VEDICINALS ™ - 9 / MOLECUSAN ™ - 9) but CORONASH ™ is considered as primary name of the test iten throughout the study.

ANNEXURE II: GLP CERTIFICATE

GOVERNMENT OF INDIA
Department of Science and Technology
National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA)

Certificate of GLP Compliance

Based on the inspection and the subsequent follow-up actions

PRADO Preclinical Research and Development Organization Private Limited
Survey No. 170/1, Punawale Road, Tathawade
Pune – 411033 (Maharashtra)

is certified capable of conducting the below-mentioned tests/studies in compliance with Organization for Economic Co-operation & Development (OECD) Principles of GLP:

- **Toxicity Studies**

The specific areas of expertise, types of chemicals and test systems are listed in annexure overleaf.

Validity: December 12, 2018 – December 11, 2021

This certificate is subject to the condition that the test facility complies with the NGCMA's Document No. GLP-101 "Terms & Conditions of NGCMA for obtaining and maintaining GLP certification by a test facility" and OECD Principles of GLP..

Certificate No. : GLP/C-127/2018
Issue Date : 12-12-2018

(Dr. Neeraj Sharma)
Head, NGCMA