DRAFT REPORT

ACUTE ORAL TOXICITY STUDY OF PROSOLUTION TABLETS IN WISTAR RATS AS PER OECD GUIDELINE NO. 425

STUDY NO: VLTO-100204

Study Completion Date: xx.xx.2010

SPONSOR

DM CONTACT MANAGMENT
100-645 TYEE ROAD, VICTORIA
BC V9A6X5, CANADA

TEST FACILITY

VEDIC LIFESCIENCES PVT. LTD.
203, MORYA LANDMARK-I,
OFF LINK ROAD, ANDHERI (W),
MUMBAI – 400 053
INDIA

STATEMENT OF COMPLIANCE

To the best of our knowledge and belief, this Study No.VLTO-100204 entitled "Acute Oral Toxicity of Prosolution Tablets in Wistar Rats as per OECD Guideline No. 425, Acute Toxic Class Method" was performed under our supervision. The objectives laid down in the study protocol were achieved.

No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

Jayesh Chaudhary
CEO, Vedic Lifesciences Pvt. Ltd.

Deepali Jadhav
Executive

CERTIFICATE

We certify that the work reported here is a true and authentic report of the study entitled, ‘‘Acute Oral Toxicity of Prosolution Tablets in Wistar Rats as per OECD Guideline No. 425, Acute Toxic Class Method”, based on the experiment conducted in one of the partnered Toxicology Laboratory Services of VEDIC LIFESCIENCES PVT LTD (B-203 Morya Landmark I, Off New Link Road, Andheri (W), Mumbai - 400 053,) India. The results presented here are faithful reflection of data collected during the study.

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QUALITY ASSURANCE STATEMENT

The Study No.: VLTO-100204, entitled “Acute Oral Toxicity Study of prosolution Tablets in Wistar Rats” has been inspected in the spirit of OECD Principles of Good Laboratory Practice {ENV/MC/CHEM (98) 17: 1997}.

This study was inspected and findings reported to Management and to the Study Director.

Inspections were performed according to the Standard Operating Procedures of the Quality Assurance Unit. The report was audited against the approved study plan and pertinent raw data and accurately reflects the raw data.

STATEMENT OF CONFIDENTIALITY

This report which contains **CONFIDENTIAL** and **PROPRIETARY** information of **DM. CONTACT MANAGEMENT** will not be disclosed to anyone except the employees of this company wherever necessary or to persons authorized by law or judicial judgment without the expressed or written approval of Sponsor.

DECLARATION

The Study Director hereby declares that the work was performed under his supervision and in accordance with the described procedures. It is assured that the reported results faithfully represent the raw data obtained during the experimental work. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study.

The Study Director accepts overall responsibility for the technical conduct of the study as well as the interpretation, analysis, documentation and reporting of the results.

1. STUDY DETAILS

1.1 TITLE : ACUTE ORAL TOXICITY STUDY OF PROSOLUTION TABLETS IN WISTAR RATS

1.2 STUDY NUMBER : VLTO-100204

1.3 TESTING FACILITY : VEDIC LIFESCIENCES PVT. LTD,
203, Morya Landmark-I,
Off Link Road, Andheri (W),
Mumbai – 400 053, INDIA

1.4 SPONSOR : DM CONTACT MANAGMENT,
100-645, Tyee Road
Victoria, Bc V9a 6x5,
Canada

1.5 STUDY SCHEDULE :

a. Study Initiation date : 10.04.2010

b. Date of procurement of animals: 10.04.2010

c. Acclimatization: Start : 10.04.2010 End: 26.04.2010

d. Treatment date : 175 mg/kg-16.04.2010,
550 mg/kg-18.04.2010,
2000 mg/kg-20.04.2010, 22.04.2010 and
24/04/2010

e. Experiment end date : 08.05.2010

f. study Completion date : xx.05.2010

2. MONITORING PERSONNEL

Sl. No.	Responsibility	Personnel	Signature with date
1.	MONITORING SCIENTIST	DEEPALI JADHAV VEDIC LIFESCIENCES PVT.LTD MUMBAI	
2.	SPONSOR'S NOMINEE	JAYESH CHAUDHARY VEDIC LIFESCIENCES PVT.LTD MUMBAI	

3. SUMMARY

The objective of this study was to assess the toxic potential and to estimate the LD50 of Prosolution tablets when administered by oral gavage in a single dose to female rats at one or more defined doses.

Single animal was dosed in sequence usually at 48hr intervals. Dosing was initiated at 175 mg/kg dose level. The starting dose was selected from the Up-and-Down procedure sequence of 1.75, 5.5, 17.5, 55, 175, 550 and 2000 mg/kg, because of unavailability of sufficient toxicological data on the test item. The test item was administered at an equivolume of 10 mL/kg body weight.

All the animals were observed for clinical signs of toxicity and mortality at 10min, 30 min, 1h, 2h, 4h and 6h following dosing and thereafter once daily during the 14 day observation period. Body weights were recorded weekly.

At the completion of study, the animals were sacrificed by CO_2 asphyxiation and subjected to detailed necropsy examination.

Treatment with Prosolution tablets up to and at 2000 mg/kg dose level was well tolerated.

There were no clinical signs and mortalities noticed in any of the doses tested. There were no changes in body weights and no gross pathological changes in any of the animals.

4.0 Conclusion

The LD50 of Prosolution tablets when administered by oral gavage was found to be >2000 mg/kg.

5. STUDY COMPLIANCE

The study was performed in accordance with the following:

a. The OECD Guidelines for Testing of Chemicals (No. 425, Section 4: Health Effects) “Acute Oral Toxicity-Up-and-Down Procedure” (Adopted: 23 March 2006).

b. In the spirit of principles of Good Laboratory Practice (1997).

c. The standard operating procedures at Vedic Lifesciences and as per the mutually agreed study plan with the sponsor.

The recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility published in the gazette of India, December 15th 1998 and the protocol approved by Institutional Animal Ethics Committee (IAEC).

6. OBJECTIVE

The objective of this study was to assess the toxic potential and to estimate the LD50 of Prosolution tablet when administered by oral gavage in a single dose to female rats at one or more defined doses.

7. MATERIALS AND METHODS

The following materials and methods were adopted for the present study:

7.1 TEST ITEM INFORMATION

The test item information as furnished by the sponsor is as follows:

Test Item name	:	Prosolution tablet
Physical appearance	:	White colored Tablets
Batch No.	:	N/A
Manufacture date	:	N/A
Expiry date	:	N/A
Storage Conditions	:	Ambient (+18 to +36^{o}C)
Name of the manufacturer & Supplier	:	**DM.CONTACT MANAGEMENT** 100-645, Tyee Road Victoria, Bc V9a 6x5, Canada

The responsibility for the correct identity and stability of the test item rests with the sponsor.

7.2 VEHICLE

Carboxymethyl cellulose (0.25%) was used as a vehicle for formulation preparation.

7.2.1 Justification for Selection of Vehicle

Carboxymethyl cellulose was commonly used as vehicle in oral toxicity studies.

7.3 TEST SYSTEM

7.3.1	**Animal species**	: Rats
7.3.2	**Strain**	: Wistar Rats
7.3.3	**Justification for selection of species**	: Rat is one of the recommended species by regulatory agencies for conducting pre clinical toxicological studies among rodents.
7.3.4	**Source**	: In-house bred animals
7.3.5	**No. of animal & sex**	: Total of 6 females were received. Females were nulliparous and non-pregnant.
7.3.6	**Body weight range at receipt**	: 142.6-150.1 g
7.3.7	**Age at treatment**	: 8 to 10 Weeks
7.3.8	**Identification**	: Ear notching and cage cards.

7.4 PERFORMANCE OF TEST

7.4.1 Husbandry

a). Conditions: Animals were housed under standard laboratory conditions, air-conditioned with adequate fresh air supply (Air changes 12-16 per hour), room temperature 21.0-24.2^{o}C relative humidity 53-65%, with 12 hours light and 12 hours dark cycle. The temperature and relative humidity was recorded once daily.

b). Housing: Maximum of three animals per dose were housed in a standard polypropylene cage (Size: L 430 x B 270 x H 150 mm) with stainless steel mesh top grill having facilities for holding pelleted food and drinking water in water bottle fitted with stainless steel sipper tube.

c). Acclimatization: The animals were acclimatized minimum of five days to laboratory conditions and observed for clinical signs daily. Veterinary examination of all the animals was performed on the day of receipt and on 5th day of acclimatization period.

d). Diet: The animals were fed *ad libitum* throughout the acclimatization and experimental period. Nutrilab rodent feed manufactured by M/S Tetragon Chemie Private Ltd, (Vetcare), Bangalore, India was provided.

e). Water: Water was provided *ad libitum* throughout the acclimatization and experimental period. Deep bore-well water passed through activated charcoal filter and exposed to UV rays in Aqua guard water filter cum purifier manufactured by Eureka Forbes Ltd., Mumbai, India was provided in plastic water bottles with stainless steel sipper tubes.

7.5 STUDY DESIGN

Single animal was dosed in sequence at 48 hr intervals. Dosing was initiated at 175 mg/kg dose level. The starting dose was selected from the OECD TG 425 Up-and-Down procedure sequence of 1.75, 5.5, 17.5, 55, 175, 550 and 2000 mg/kg because of unavailability of sufficient toxicological data on the test item.

Dosing was sequential, allowing at least 48 hours before dosing the next animal. The time interval between dosing at each level was determined by the onset, duration, and severity of toxic signs. There was no moribundity or mortality at starting dose of 175 mg/kg, hence further animals were treated at the next higher doses with single animal at 550 and three animals at 2000 mg/kg as per the OECD TG 425 Up-and-Down procedure.

The dosing sequence was followed using self-contained software for OECD Guideline 425 “AOT425Statpgm”. Dose levels higher than 2000 mg/kg were not tested.

The testing stopping criterion was following when the first 3 consecutive animals survive at the upper bound.

The estimated LD50 was calculated from the animal outcomes at test termination using self-contained software for OECD Guideline 425 “AOT425Statpgm”.

7.6 DOSE FORMULATION

The test item was finely grinded in a mortar with the help of pestle and required quantity was weighed as per the doses 175, 550 and 2000 mg/kg used in the study and suspended in 0.25% Carboxymethyl cellulose to get desired concentration of 17.5, 55 and 200 mg/mL respectively. Formulation of the test item was prepared shortly before dosing.

7.7 ADMINISTRATION OF TEST ITEM

The animals were fasted overnight prior to dosing. Water was provided during fasting period. The test item was administered by oral gavage to each rat as a single dose, using gavaging needle. The dosage volume administered to individual rat was adjusted according to its body weight recorded on the day of dosing. The

dose volume was 10 mL/kg body weight for all animals. Food was offered 3-4 hours followed by dosing.

8. OBSERVATIONS

The following observations were undertaken during the study.

8.1 CLINICAL SIGNS AND PRE-TERMINAL

All the animals were observed for clinical signs and mortality at 10-15min, 30-40min, 1hr (±10min), 2hr (±10min), 4hr (±10min) and 6hr (±10min) on day 1 following dosing and thereafter once daily, for a total of 14 days observation period. Duration of observations was determined by the toxic reactions, rate of onset and length of recovery period. The appearance, change and disappearance of these signs were recorded.

8.2 BODY WEIGHTS

Individual animal body weights were recorded on day 1 before test item administration and on day 7 and 14 during the study period.

8.3 PATHOLOGY

At the completion of the study period, the animals were subjected to following pathological examinations.

8.3.1 Necropsy

At the end of each animal observation period, animals were sacrificed by CO_2 asphyxiation and subjected to a complete necropsy.

8.3.2 Histopathology

Histopathological examination was not carried out as there were no gross pathological changes noted at necropsy.

9. DATA COMPILATION

The computer printout of the data (in the form of appendix) was verified with the original raw data by the study group. The data on body weight and weight changes were subjected to computer statistical processing wherever possible. All individual animal data was summarized and presented as tables. All findings were presented in the report as per the standard operating procedure.

10. ARCHIVING

All test article, raw data and other documents generated during the course of this study together with a copy of final report will be stored in the archives of Vidic Lifesciences, Mumbai, India for a period of one year from the date of submission of final report.

11. RESULTS AND DISCUSSION

11.1 CLINICAL SIGNS AND MORTALITY

There were no clinical signs of toxicity and mortalities noticed in dose tested.

Refer Table - 1 and Appendix – 1

11.2 BODY WEIGHTS

There were no changes in body weight and percent body weight gain was noted over the study period at all the doses tested.

Refer Table - 2 and Appendix - 2

11.3 PATHOLOGY

There were no external and internal gross pathological lesions noticed in any of the animals sacrificed at the end of the study.

Refer Table -3 and Appendix -3

12. CONCLUSION

The LD50 of Prosolution tablet when administered by oral gavage was found to be >2000 mg/kg.

TABLE - 1

SUMMARY OF CLINICAL SIGNS AND MORTALITY

Refer Appendix – 1

Dose (mg/kg)	No. of Animals	Sex	Clinical signs	Mortality
175	1	Female	NAD	0/1
550	1	Female	NAD	0/1
2000	3	Female	NAD	0/3

NAD: No Abnormality Detected

TABLE - 2

SUMMARY OF BODY WEIGHTS (g) AND BODY WEIGHT GAIN (%)

Refer Appendix - 2

Dose (mg/kg)	No. of Animals	Sex		Body weights on day				% Body weight gain	
				1	7	14		1-7	1-14
175	1	Female		150.60	168.10	175.30		11.62	16.40
550	1	Female		159.40	169.30	179.80		8.46	15.18
2000	3	Female	Mean	161.63	184.23	196.83		14.01	21.80
			± SD	±2.42	±2.72	±1.40		±3.31	±2.27

TABLE - 3

SUMMARY OF GROSS NECROPSY FINDINGS

Refer Appendix - 3

Dose (mg/kg)	No. of Animals	Sex	Necropsy findings	
			External	Internal
175	1	Female	NAD	NAD
550	1	Female	NAD	NAD
2000	3	Female	NAD	NAD

NAD: No Abnormalities Detected

APPENDICES

APPENDIX - 1

INDIVIDUAL ANIMAL CLINICAL SIGNS AND MORTALITY RECORD

Dose (mg/kg)	Animal No.	Sex	Study Day 1						Study Days												
			10 m	30 m	1h	2h	4h	6h	2	3	4	5	6	7	8	9	10	11	12	13	14
175	1	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
550	2	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2000	3	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	4	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	5	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N: Normal, F: Female

APPENDIX – 2

INDIVIDUAL ANIMAL BODY WEIGHTS (g) AND BODY WEIGHT GAIN (%)

Dose (mg/kg)	Animal No.	Sex	Body weights on day				% Body weight gain	
			1	7	14		1-7	1-14
175	1	F	150.6	168.1	175.3		11.6	16.4
550	2	F	156.1	169.3	179.8		8.5	15.2
	3	F	159.4	185.9	196.4		16.6	23.2
2000	4	F	161.3	185.7	198.4		15.1	23.0
	5	F	164.2	181.1	195.7		10.3	19.2

F: Female

APPENDIX - 3

INDIVIDUAL ANIMAL GROSS NECROPSY FINDINGS

Dose (mg/kg)	Animal No.	Sex	Type of Death	Necropsy findings	
				External	Internal
175	1	F	TS	NAD	NAD
550	2	F	TS	NAD	NAD
	3	F	TS	NAD	NAD
2000	4	F	TS	NAD	NAD
	5	F	TS	NAD	NAD

NAD: No Abnormalities Detected, TS: Terminal Sacrifice