STUDY REPORT	
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TEST ITEM: "**PDBGR34 (NBRMAP-DB)**"	STUDY NO.: **SDPARC/AFC/TOX/14/06**

STUDY TITLE

SINGLE DOSE ORAL ACUTE TOXICITY STUDY OF "PDBGR34 (NBRMAP-DB)" IN WISTAR RATS

SPONSOR

Aimil Pharmaceuticals (I) Ltd.
Pillar No-208, Main Patel Road,
Patel Nagar, New Delhi,
Delhi 110008, India
Tel. Work: +91-11-25705472
Mob: +91-+91-9999108284
Web: www.aimilpharmaceuticals.com

TEST FACILITY

Shree Dhanvantary Pharmaceutical Analysis & Research Centre (SDPARC)
Kim (East), Surat,
Gujarat-394110, India
Tel.: +91-9924304830, +91-9904155567
Email:afc@sdparc.com

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COMPLIANCE STATEMENT

The Study Director hereby declares that the work was performed under his supervision and in accordance with the mutually agreed study plan and the standard operating 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.

Date: 30/09/2014

Study Director

ABBREVIATIONS USED

Abbreviation	Description
G	Gram
Kg	Kilogram
%	Percent / percentage
Mg	Milligram
No.	Number
Sec	Seconds
mL	Milliliter
µl	Microliter
CMC	Carboxy methyl cellulose
OECD	Organization for economic cooperation and development
TG	Test Guideline
DRS	Data Recording Sheet

1. STUDY DETAILS

Study Title : Single dose oral acute toxicity study of "PDBGR34 (NBRMAP-DB)" in Wistar Rats

Study No. : SDPARC/AFC/TOX/14/06

Test Item : "PDBGR34 (NBRMAP-DB)"

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Study Director : Mr. Gagan Goswami

Study Scientists : Mr. Santosh

Study Schedule

Study initiation Date : 18/08/14

Experimental Start Date : 25/08/14

Experimental Completion Date : 11/09/14

Study Completion Date : 30/09/14

2. STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name	Responsibility	Signature (With date)
Mr. Gagan Goswami (Study Director)	Overall incharge for the planning, conduct and report preparation of the study	G. Goswami 30/09/2014
Mr. Santosh (Study Scientist)	Assist in conduct of study	Santosh 30/09/2014

3. SUMMARY

Objective of the present study was to evaluate the single dose acute oral toxicity of the test item "PDBGR34 (NBRMAP-DB)" by following OECD test guideline 425.

As per the OCED TG 425 guideline, 15 adult female wistar rats of similar age and body weight were taken for the experiment. Animals were acclimatized in standard animal house environmental conditions for 7 days before the start of experiment.

All animals were dosed orally once in a stepwise manner i.e. next higher or lower dose level was administered to next animal after observation of previous animal for any mortality / evident toxicity for 48 hrs. Dose levels were progressed in geometric progression with a factor of 2.

Dosing was started by oral administration of 250 mg/kg bw of test item to 1st animal. As no toxicity / mortality was observed in 1st animal when observed for 48 hrs, next animal was treated with 250 mg/kg bw dose and observed in a similar manner to 1st animal and so on. As no mortality was observed at the limit test dose level of 2000 mg/kg bw, additional four animals were dosed sequentially at the same dose level so that a total of 5 animals were tested as per the guidelines.

None of the "PDBGR34 (NBRMAP-DB)" treated animals showed any significant decrease in body weight as compared respective pre-treatment values during the observation period.

All animal were observed daily during the treatment period for any clinical signs of toxicity. Animal treated with "PDBGR34 (NBRMAP-DB)" did not show any clinical sign of toxicity during the observation period.

No mortality was observed in any of the "PDBGR34 (NBRMAP-DB)" treated animal even at the limit test dose level of 2000 mg / kg bw. Necropsy was not performed as no morbidity and mortality was observed during the observation period.

From the findings of the study it can be concluded that Test item "PDBGR34 (NBRMAP-DB)" is non toxic in single dose oral acute toxicity study at the limit test dose level of 2000 mg/kg bw. Further, as no toxicity was found at limit test dose level, median LD_{50} of the product can be concluded as above 2000 mg / kg bw.

4. OBJECTIVE

To evaluate the single dose oral acute toxicity of test item **"PDBGR34 (NBRMAP-DB)"** in Wistar rats.

5. SAFETY PRECAUTIONS

Personal protection equipments like gloves, masks, aprons, footwear were employed as required while handling the test item and test system.

6. MATERIALS AND METHODS

6.1 TEST ITEM INFORMATION

6.1.1 Test Item description

Name of the test item	:	"PDBGR34 (NBRMAP-DB)"
Appearance	:	Dark Brownish bi-convex Tablets
Batch No.	:	PD-BGR34-01
Mfg. Date	:	August, 2014
Exp. Date	:	July, 2017

6.1.2 Test Item Analysis

Analysis for the identity and purity of the test item was not conducted as part of this study, and is the responsibility of the sponsor.

6.2 TEST SYSTEM

Species		*Rattus norvigicus*
Strain		Wistar
Source		Animal Facilitation Centre
Sex		Female
Source		Mahaveera Enterprises, Hyderabad
Number of animals		12

7. EXPERIMENTAL DESIGN AND PROCEDURE

7.1 PRINCIPLE OF TEST METHOD [1]

The protocol was designed to investigate the single dose acute oral toxicity of **"PDBGR34 (NBRMAP-DB)"** in female Wistar rats as per OECD Test Guideline 425.

Animals are dosed, one at a time, at 48 hour intervals. The first animal receives a dose at the level of the best estimate of the LD_{50}. Depending on the outcome for the previous animal, the dose for the next animal is adjusted up or down. If an animal survives, the dose for the next animal is increased; if it dies, the dose for the next animal is decreased. After reaching the reversal of the initial outcome, i.e. the point where an increasing (or decreasing) dose pattern is reversed by giving a smaller (or a higher) dose, four additional animals are dosed following the same UDP. The LD_{50} is calculated using the method of maximum likelihood.

Limit Test: Dose one animal at the test dose. If the animal dies, conduct the main test to determine the LD50. If the animal survives, dose four additional animals sequentially so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. The LD50 is greater than 2000 mg/kg if three or more animals survive. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. Late deaths should be counted the same as other deaths.

7.2 DESCRIPTION OF THE TEST PROCEDURE [1]

15 adult female wistar rats of similar age and body weight were taken for the experiment. Animals were acclimatized in Standard Animal House environmental conditions for 7 days before the start of experiment. All animals were dosed orally once in a stepwise manner i.e. next higher or lower dose level was administered to next animal after observation of previous animal for any mortality / evident toxicity for 48 hrs. Dose levels were progressed in geometric progression with a factor of 2.

Dosing was started by oral administration of 250 mg/kg bw of test item to 1st animal. As no toxicity / mortality was observed in 1st animal when observed for 48 hrs, next animal was treated with 250 mg/kg bw dose and observed in a similar manner to 1st animal and so on. Dosage progression has been depicted in table 1. As no mortality was observed at the limit test dose level of 2000 mg/kg bw, additional four animals were dosed sequentially at the same dose level so that a total of 5 animals were tested as per the guidelines.

Table 1: Dosage progression for LD_{50} determination of "PDBGR34 (NBRMAP-DB)"

Day	Animal no.	Dose (mg/kg bw)	Outcome
1	1 (H)	250	No death
3	2 (B)	500	No death
5	3 (T)	1000	No death
7	4 (HB)	2000*	No death
9	5 (BT)	2000*	No death
11	6 (HT)	2000*	No death
13	7 (HBT)	2000*	No death
15	8 (UM)	2000*	No death

'* 2000 mg/kg bw is the limit test dose

All animals were observed for atleast 7 days for Clinical signs of toxicity, Body weight change and mortality during the observation period and data was recorded in controlled DRS.

7.2.1 Preparation of Test items

Test item was prepared as 25, 50, 100 and 200 mg / mL solution in 0.1 % sodium CMC solution.

7.3 JUSTIFICATION FOR SELECTION OF ROUTE OF ADMINISTRATION

Oral route is the intended route for test item administration as suggested by the sponsor.

Acclimatization		7 days
Identification of animals		**Pre-randomization-** Temporary tail marking with marker; **Post-randomization-** Permanent body marking; **Cage Labeling-** Labeling with complete study details
Randomization		Animals were randomly selected based upon their body weight and allotted to different groups.

6.3 JUSTIFICATION FOR SELECTION OF TEST SYSTEM

Wistar rats were selected as the Test System as it is commonly reported in literature (OECD Test Guidelines 425) for the evaluation of acute oral toxicity study of test item [1].

6.4 ANIMAL HUSBANDRY

Temperature	:	22 ± 3 °C
Relative humidity	:	30 to 70%
Housing	:	Standard polypropylene rats cages with stainless steel top grill were used to house the animals. The cages were washed and cleaned paddy husk was used as the bedding material.
Sanitation	:	Bedding material was changed as per the Standard Operating Procedure.
Light/dark cycle	:	12-hourly
No. of animals per cage	:	5
Feed & water	:	Standard pelleted feed was provided *ad libitum*. Filtered water was provided *ad libitum*. The diet and water was routinely analyzed for any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

8. OBSERVATIONS

8.1 BODY WEIGHT

Body weight of all experimental animals was recorded weekly twice during the study period.

8.2 CLINICAL SIGNS OF TOXICITY

General clinical observations were done daily, preferably at the same time(s) each day. The health condition of the animals was recorded in corresponding data recording sheets (DRS).

8.3 MORTALITY

All animals were closely observed during first 4 hours after dosing followed by atleast twice daily.

8.4 GROSS NECROPSY

Necropsy was not performed as no morbidity and mortality was observed during the observation period.

9. RESULTS

9.1 BODY WEIGHT

None of the PDBGR34 (NBRMAP-DB) treated animals showed any significant decrease in body weight as compared respective pre-treatment values during the observation period.

9.2 CLINICAL SIGNS OF TOXICITY

All animal were observed daily during the treatment period for any clinical signs of toxicity. Animal treated with "PDBGR34 (NBRMAP-DB)" did not show any clinical sign of toxicity during the observation period.

9.3 MORTALITY

No mortality was observed in any of the "PDBGR34 (NBRMAP-DB)" treated animal even at the limit test dose level of 2000 mg / kg bw.

9.4 GROSS NECROPSY

Necropsy was not performed as no morbidity and mortality was observed during the observation period.

10. DISCUSSION & CONCLUSION

Objective of the present study was to evaluate the single dose acute oral toxicity of the test item "PDBGR34 (NBRMAP-DB)" by following OECD test guideline 425.

As per the OCED TG 425 guideline, 15 adult female wistar rats of similar age and body weight were taken for the experiment. Animals were acclimatized in standard animal house environmental conditions for 7 days before the start of experiment.

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11. REFERENCES

OECD guidelines Test Guideline 425

12. REPORT DISTRIBUTION

A copy of the draft report will be sent for Sponsor's approval.

The final report (original copies) will be distributed as follows:

Sponsor: One signed final report in original

Test Facility: One signed final report in original

13. ARCHIVES

The study plan, raw data and final report will be archived at the Test Facility for 2 years after completion of the study.