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	STUDY NO.: SDPARC/AFC/TOX/16/15

STUDY TITLE

**28-DAYS REPEATED DOSE SUB-ACUTE ORAL TOXICITY STUDY OF "BGR 34" IN
WISTAR RAT**

SPONSOR

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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.

Study Director

Date:



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ABBREVIATIONS USED

Abbreviation	Description
G	Gram
Kg	Kilogram
%	Percent / percentage
Mg	Milligram
No.	Number
Sec	Seconds
mL	Milliliter
µl	Microliter
NOAEL	No observed adverse effect level
OECD	Organization for economic cooperation and development
TG	Test Guideline
DRS	Data Recording Sheet



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1. STUDY DETAILS

Study Title : 28-days repeated dose Sub-acute Oral toxicity study of "BGR 34" in Wistar Rats.

Study No. : SDPARC/AFC/TOX/16/15

Test Item : "BGR 34"

Sponsor : **Aimil Pharmaceuticals (I) Ltd.**
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Web: www.aimilpharmaceuticals.com

Study Director : Mr. Gagan Goswami

Study Scientists : Krutika Gamit

Study Schedule

Study initiation Date : 23/11/2016

Experimental Start Date : 6/12/2016

Experimental Completion Date : 16/01/2017

Study Completion Date : Within one week of sponsor's approval to final report



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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	
Ms. Krutika Gamit (Study Scientist)	Assist in conduct of study	



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3. OBJECTIVE

To evaluate 28-days repeated dose sub-acute oral toxicity study of “BGR 34” in Wistar rats.

4. SAFETY PRECAUTIONS

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

5. MATERIALS AND METHODS

5.1 TEST ITEM INFORMATION

5.1.1 Test Item description

Name of the test item	:	“BGR 34”
Appearance	:	Dark brown Tablets
Batch No.	:	NBGR-564-H
Mfg. Date	:	JULY-2016
Exp. Date	:	JULY-2019
pH	:	
Composition of Test Item		
Ingredient	CAS	Quantity
Daruhrida(berberis aristata,st.)	1150 mg	75mg
Vijaysar(pterocarpus marsupium)	400mg	25mg
Gymnema sylvestre lf.	400mg	25mg
Majeeth(rubia cordifolia,rt)	375mg	25mg
Methika(trigonella foenum-graecum,sd.)	350mg	25mg
Giloy(tinospora cordifolia,st)	350mg	25mg

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Shlajeet sudh	10mg	
Sodium Methyl Paraben		Traces
Sodium Propylparaben		Traces

5.1.2 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.

5.2 TEST SYSTEM

Species	<i>Rattus norvigicus</i>
Strain	Wistar
Source	Animal Facilitation Centre
Sex	Male & Female
Source	Mahaveera Enterprises, Hyderabad
Number of animals	50 animals (25 male + 25 female)
No. of animals / group	5 animals per group
Acclimatization	15 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.

5.3 JUSTIFICATION FOR SELECTION OF TEST SYSTEM

Wistar rats were selected as the Test System as it is commonly reported in literature (OECD Test Guidelines 407) for the evaluation of repeated dose sub-acute oral toxicity study of test item ¹.

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5.4 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 (Male and female rats were housed in separate cages)
Feed & water	:	Standard pelleted feed was provided <i>ad libitum</i> . Filtered water was provided <i>ad libitum</i> . The diet and water was routinely analyzed for any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

6. EXPERIMENTAL DESIGN AND PROCEDURE

6.1 PRINCIPLE OF TEST METHOD ⁽¹⁾

The protocol was designed to investigate the 28-Days repeated dose oral toxicity of BGR 34 in male and female Wistar rats as per OECD Test Guideline 407.

As per the guideline, the test substance is orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 28 days. During the period of administration the animals are observed closely, each day for signs of toxicity. Animals that die or are euthanised during the test are necropsied and at the conclusion of the test surviving animals are euthanised and necropsied. A 28 day study provides information on the

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effects of repeated oral exposure and can indicate the need for further longer term studies. It can also provide information on the selection of concentrations for longer term studies. The data derived from using the TG should allow for the characterization of the test substance toxicity, for an indication of the dose response relationship and the determination of the No-Observed Adverse Effect Level (NOAEL).

6.2 DESCRIPTION OF THE TEST PROCEDURE ⁽¹⁾

50 adult Wistar rats (25 males + 25 females) were taken for the experiment. Animals were acclimatized in Standard Animal House environmental conditions for 15 days before the start of experiment. On the last day of acclimatization, animals were randomized and allocated to 5 groups viz. G1, G2, G3, G4 & G5. Male and female animals were randomized and grouped separately.

G1 served as vehicle control group treated in a similar manner as treatment group except the administration of Test item. G2, G3 and G4 served as high, intermediate and low dose groups, treated with 1500, 750 & 375 mg/kg bw/day of test item respectively given orally.

G5 served as satellite group which was treated in a manner similar with high dose level. All test group animals were dosed with the test item daily seven days each week for a period of 28 days. Animals were allocated to groups of animals as mentioned in table below:

6.2.1 Allocation of Animals

Group	Treatment	Dose (mg/kg bw /day)	Route of administration	No. of animals / group
G1	Vehicle control*	-	p.o.	5 male + 5 female
G2	High dose	1500		5 male + 5 female
G3	Mid dose	750		5 male + 5 female
G4	Low dose	375		5 male + 5 female
G5	Satellite	1500		5 male + 5 female

*Vehicle control group will be treated in a similar manner as treatment group except the administration of Test item



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All animals were observed for 28 day except animals in satellite group which were subjected to observations for further 14 days without treatment to detect delayed occurrence, or persistence of, or recovery from toxic effects.

G1, G2, G3 & G4 groups were sacrificed after dosing for 28 days. Necropsy was done to observe any gross changes, blood was collected to analyze biochemical and hematological parameters and major organs were collected to observe any histological changes.

Clinical signs of toxicity, Body weight change and mortality were recorded daily during the observation period in controlled DRS.

6.2.2 Preparation of Test items

Test item was dissolved in purified water to prepare 15, 7.5 and 3.75 g/mL solution for dosing.

6.3 JUSTIFICATION FOR SELECTION OF ROUTE OF ADMINISTRATION

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

7. OBSERVATIONS

7.1 BODY WEIGHT

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

7.2 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).

7.3 HEMATOLOGY

On the end of dosing and observation period, blood was collected from retroorbital sinus from all animals and following haematological examinations were performed at the end of the test period: haematocrit (%), haemoglobin (gm%), Total leukocyte count (TLC), Differential leukocyte count (DLC).



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7.4 CLINICAL BIOCHEMISTRY

Clinical biochemistry determinations to investigate major toxic effects in tissues and, specifically, effects on kidney and liver, were performed on blood samples obtained from all animals just prior to killing the animals. Overnight fasting of the animals prior to blood sampling was done. The following biochemical examinations were performed at the end of the test period: Protein (gm/dl), Glucose (mg/dl), Urea (mg/dl), Creatinine (mg/dl), SGPT (U/L), SGOT (U/L), ALP (IU/L).

7.5 GROSS NECROPSY

Necropsy was performed for animals in severe distress / morbid condition / animal died during dosing and on study termination to observe any test item related toxicity.

Necropsy was performed for all animals after 28 days repeated dosing except satellite group which was observed for further 14 days. All animals were observed for any gross pathological changes. After examination of external appearance of the cranial, thoracic, and abdominal cavities were opened and the appearance of the tissues and organs was observed.

All animals in the study were subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

7.6 RELATIVE ORGAN WEIGHT

The brain, heart, adrenal, testes (in male animals), ovary (in female animals), epididymis, uterus, kidneys, liver, thymus, lungs, spleen and GI tract of all animals were weighed wet as soon as possible after dissection to avoid drying.

8. RESULTS

8.1 BODY WEIGHT

All male and female animal groups showed gradual significant increase in body weight during the observation period.



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8.2 CLINICAL SIGNS OF TOXICITY

All animal were observed daily during the treatment period for any clinical signs of toxicity. 3 male and 2 female animals of G2 (high dose group) and G5 (Satellite group) showed piloerection in third week of treatment. G1 (Vehicle control), G3 (intermediate dose) and G4 (Low dose group) did not show any clinical signs of toxicity during the study period.

Reversal in piloerection was observed in all male and female animals of G5 (satellite group) during the observation period of additional 14 days post treatment.

8.3 HEMATOLOGY

Haemoglobin (Hb)

The haemoglobin level did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

TLC

The WBC count did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

Neutrophil

No significant decrease in neutrophil count as compared to background normal value was observed in any of treated group.

Lymphocyte

The lymphocyte count did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

Monocyte

The Monocyte count did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

Eosinophil

The Eosinophil count did not show any significant variation and values were found to be within normal limits in all control/treatment groups in male as well as female animals.



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Basophil

The Basophil count did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

8.4 CLINICAL BIOCHEMISTRY

SGPT (U/L)

SGPT level in the treatment groups did not show any significant variation as compared to respective control group.

SGOT (U/L)

SGPT level in the treatment groups did not show any significant variation as compared to respective control group.

Alkaline phosphatase (ALP)

ALP level in the treatment groups did not show any significant variation as compared to respective control group.

Bilirubin

Bilirubin level did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

Urea

Blood urea level did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

Creatinine

Creatinine level did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

Glucose

Glucose level did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

Albumin



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Albumin level did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

Total serum protein

Total serum protein level did not show any significant variation and values were found to be within normal limits in all control/treatment groups.

Data has been expressed as Mean \pm SEM

8.5 GROSS NECROPSY

Gross necropsy was performed for G1, G2, G3, & G4 on experiment termination i.e. after treatment for 28 days. G5 (satellite group) was observed for further 14 days without test item administration.

Mild pin point hemorrhage in lungs and pneumonitis was observed in 1 female animal of G2 (high dose treated group)

No macroscopical abnormality was found in any of observed organs / tissues of treated groups.

8.6 HISTOPATHOLOGY

No abnormality was seen in any of the treated groups of high dose treated group as compared to background vehicle control. Hence, histopathological analysis was not extended to other groups.

9. DISCUSSION & CONCLUSION

Objective of the present study was to evaluate the 28-days repeated dose sub-acute oral toxicity of Test Item “BGR-34”. The study was performed by following OECD test guideline 407.

As per the OCED TG 407 guideline, 50 adult Wistar rats (25 males + 25 females) were taken for the experiment. Animals were acclimatized in Standard Animal House environmental conditions for 15 days before the start of experiment. On the last day of acclimatization, animals were randomized and allocated to 5 groups viz. G1, G2, G3, G4 & G5. Male and female animals were randomized and grouped separately.



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G1 served as vehicle control group treated in a similar manner as treatment group except the administration of Test item. G2, G3 and G4 served as high, intermediate and low dose groups, treated with 1500, 750 & 375 mg/kg bw/day of test item respectively given orally daily for 28 days.

G5 served as satellite group which was treated in a manner similar with high dose level. All test group animals were dosed with the test item daily seven days each week for a period of 28 days.

Clinical signs and mortality was observed daily. Fasting blood was collected from all animals on last day of treatment to perform hematological analysis and serum biochemistry. Necropsy was performed at the end of experiment to observe any gross pathological findings and wet organ weight was recorded.

All male and female animal groups showed gradual significant increase in body weight during the observation period.

All animal were observed daily during the treatment period for any clinical signs of toxicity. 3 male and 2 female animals of G2 (high dose group) and G5 (Satellite group) showed piloerection in third week of treatment. G1 (Vehicle control), G3 (intermediate dose) and G4 (Low dose group) did not show any clinical signs of toxicity during the study period.

Reversal in piloerection was observed in all male and female animals of G5 (satellite group) during the observation period of additional 14 days post treatment.

One mortality was found in male animal of G3 (intermediate dose group).

No abnormality and significant variation was observed in Hematological and Biochemical parameters in any of control/treated group animals.



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No macroscopical abnormality was found in any of observed organs / tissues of G1 (Control) and treated groups except mild pin point hemorrhage in lungs and pnemonitis was observed in 1 female animal of G2 (high dose treated group).

No microscopic abnormality was seen in any of the analysed organs of high dose treated groups as compared to background vehicle control. Hence, histopathological analysis was not extended to other groups.

In light of above mentioned observations, it can be concluded that Test item “BGR-34, at the dose level of 1500 mg/kg bw is not associated with any pre-clinical toxicities when given orally for 28 days repeated exposure period. Therefore, 1500 mg/kg bw dose level is determined as No-Observed Adverse Effect Level (NOAEL) for 28 days repeated oral dose toxicity study of BGR-34.

10. REFERENCES

OECD guidelines Test Guideline 407

11. 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

12. ARCHIVES

The study plan, raw data and final report will be archived at the Test Facility for 5 years after completion of the study.