Mahatma Gandhi Medical College & Research Institute,
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Department of Pharmacology
Repeated dose 28 days toxicity study of Clevira syrup in Wistar rats

Name of the Investigator: Dr Uma Narayanamurthy
Assistant Professor, Department Of Pharmacology

Place of study: Central Animal House,MGMC&RI,Pondicherry

Sponsor: Apex Laboratories, Guindy, Chennai

Test substance(Product): Syrup Clevira

Species : Male(30) and Female(30) Wistar rats, total 60 nos, 10-12 weeks

Assay: Repeated Dose Toxicity Study

Project Manager: Dr.M.Sakthi Balan, Investigator, ki3.

CRO: Ki3, Chennai.

Clevira is a herbal preparation from Apex laboratories, Chennai. Clevira is available in syrup and tablet form. Clevira preparations are indicated for the treatment of viral infections as it is claimed for its antiviral property. The in-vitro cytotoxic studies and antiviral activity assays in cell culture has proven its activity. (1)

In this study as an initial step of preclinical study we have assessed the repeated dose toxicity status of the drug. This study was done in accordance with the OECD guidelines for toxicity studies and after approval from the Institutional Animal Ethics committee (05/IAEC/MGMC/11/2018-II).

CLEVIRA SYRUP COMPOSITION

Each 10 ml contain (Aushadh Ghana) extracts derived from medicinal plants of

S. No.	Botanical Name	Common Name (Sanskrit Name)	Plant Parts Used	Label Claim (mg)
1.	Carica papaya	Erandakarkati	Leaves	1000.00
2.	Melia azedarach	Mahanimba	Leaves	1000.00
3.	Andrographis paniculata	Kalmegh	Herb	250.00
4.	Vetiveria zizanioides	Usira	Root	250.00
5.	Trichosanthes dioica	Patola	Whole Plant	250.00
6.	Cyperus rotundus	Musta	Rhizome	250.00
7.	Zingiber officinale	Sunthi	Rhizome	250.00
8.	Piper nigrum	Maricha	Fruit	250.00
9.	Mollugo cerviana	Grismachatraka	Whole Plant	250.00
10.	Tinospora cordifolia	Guduchi	Stem	250.00

Ethics Committee Approval Certificate:

Sri Balaji Vidyapeeth (Deemed to be University)

ACCREDITED BY NAAC WITH 'A' GRADE

Mahatma Gandhi Medical College & Research Institute

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

MEMBERS OF IAEC

Dr. Manimekalai.K
Chairperson of IAEC & Scientist Incharge of Animal House Facility

Dr. Uma Narayanamurthy
Member Secretary IAEC & Bioloigical Scientist

Dr. A. Anita
Cpcsea Main Nominee

Dr. V. Arul
CPCSEA Link Nominee

Dr. C. Vijayalakshmi
Socially Aware Nominee

Dr. R. Barathidasan,
Scientist from outside The Institute

Dr. Jeneth Berlin Raj
Scientist from different discipline

Dr. Pramodhini S
Scientist from different discipline

CERTIFICATE OF APPROVAL

IAEC Number	05/IAEC/MG/11/2018 - II		
Strain & Species	**Wistar Rats**	**Total No. Approved**	**30 Male rats 30Female Rats**
Date of Issue	**12-11-2018**	**Date of Expiry**	**12-11-2019**

This is to certify that the project/study entitled

Repeated Dose 28 days Oral Toxicity Study Of Clevira Syrup in Wistar Rats

Submitted by Dr. Uma Narayanamurthy, Assistant Professor, Department of Pharmacology has been reviewed by the Members of IAEC, MGMCRI.

The proposal has been	☑ **Sanctioned**	☐ **Rejected**	☐ **Sanctioned with modification**

Name of Chairman/Member Secretary IAEC:	**Dr. Manimekalai.K**
Signature with Date:	K. Mani 12/11/18.
Name of CPCSEA Nominee :	**Dr. A. Anita**
Signature with Date:	Anita 12/11/2018.

Objectives:

- To assess the Repeated dose 28 days toxicity of clevira syrup in wistar rats
- To observe for signs of toxicity throughout the study period (28 days)
- To check for gross pathological changes of the major internal organs at the end of study after necropsy(on 28th day)

Outcome parameters

- Change in weight of the animals from baseline, day 7, day 14, day 21 and day 28
- Signs of toxicity
- Gross pathological changes at the end of the study

METHODOLOGY:

Repeated Dose Toxicity Study:

Following the acute oral toxicity (2000mg/kg) study(results are furnished with form B), three descending doses 1000mg/kg, 500mg/kg and 250mg/kg were selected and administered for 3 groups of 10 rats each. The other group of 10 rats served as control group. Following the period of fasting, the animals were weighed and test substance was administered. After the test substance has been administered food was withheld for 3-4 hours. The animals were dosed with test substance daily for a period of 28 days. The maximum volume of liquid that can be administered at one time will not exceed 1 ml/100g body weight.

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 28 days. All observations are systematically recorded with individual records being maintained for each animal.

Outcome parameters

- Change in weight of the animals
- Signs of toxicity is monitored
- Gross pathological changes and microscopy

Weight changes were calculated and recorded weekly. Measurements of food consumption were made at least weekly. Histopathology was done at the end of 28 days and the samples were collected as part of the procedure after euthanasia of the animals by Inj. Thiopentone sodium i.p, and stored under appropriate conditions. Animals were fasted overnight prior to euthanasia.

Table 1:

Study	Test Drug	Groups	Dose mg/kg	Species/ Strain	No. Of Animals and Sex	Total Animals
Repeated Dose Toxicity Study	Clevira Syrup	Control	Sterile water 1ml/100g	Wistar Rats	5 Male rats + 5 Female rats	10
		Low Dose	250mg/kg	Wistar Rats	5 Male rats + 5 Female rats	10
		Medium Dose	500mg/kg	Wistar Rats	5 Male rats + 5 Female rats	10
		High Dose	1000mg/kg	Wistar Rats	5 Male rats + 5 Female rats	10

Satellite Group				
Control Group	Sterile Water 1ml/100g	Wistar Rats	5 Male rats + 5 Female rats	10
High Dose of Clevira Syrup	1000mg/kg	Wistar Rats	5 Male rats + 5 Female rats	10
				60 Wistar Rats

All animals in the study shall be 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. All gross pathological changes will be recorded for each animal.

Note: All the above are followed as per the OECD test guideline 407.

RESULTS:

Table 1: Weight changes in wistar rats baseline, day 7, day 14, day 21 and day 28

Group designation	Baseline weight in kg	Weight on Day 7	Weight on Day 14	Weight on Day 21	Weight on Day 28
Test 1(n=5) Dose of Clevira syrup 250mg/kg	186+195+ 180+ 182+175 183.6	178+185+ 188+ 176+176 180.6	167+195+ 162+ 178+172 174.8	176+187+ 167+ 189+179 179.6	187+176+ 188+ 186+180 183.4
Test 2 (n=5) Dose of Clevira syrup 500mg/kg	186+179+ 174+ 176+176 178.2	178+176+ 180+ 187+180 180.2	176+183+ 172+ 170+180 176.2	180+172+ 173+ 174+174 174.6	175+170+ 170+ 172+168 171

Table 2: Observation on signs of toxicity

DAYS	0	7	14	21	28
Test 1 (250mg/kg)	Nil	Nil	Nil	Nil	Nil
Test 2 (500mg/kg)	Nil	Nil	Nil	Nil	Nil

Observation:

The animals from each group were monitored from day one of the study and the observations were done on the external features for signs of toxicity. All systems were carefully examined and recorded on individual basis on every seventh day (Day 0, 7, 14, 21 and 28 days). During the study period none of the animals showed signs of toxicity or had a moribund status in the dose of 250mg/kg and 500mg/kg, but to mention the dose of 1000mg/kg started to show the signs of toxicity from Day 15 onwards with conjunctival and paw edema, abdomen distension and hemoptysis, and mortality occurred within day 27 of all the animals in the group.

At the end of the study animals were sacrificed and necropsy was done. There was no gross pathological changes noted in group 1 (250mg/kg) and group 2 (500mg/kg). All the orifices, cranial, thoracic, pelvic and abdominal cavity and the internal organs including the reproductive organs were healthy, no signs of toxicity and necrosis of tissues were identified in them. On Histopathological examination, the liver, kidney, stomach, heart, adrenals, ovary, uterus, testes and pancreas were found to be normal without any signs of inflammation at the dose of 250mg/kg and 500mg/kg of Clevira Syrup.

Conclusion: Hence, we can conclude that syrup Clevira in doses of 250 and 500 mg/kg were non- toxic on repeated dose administration, as per OECD guidelines.

REFERENCES

1. In vitro cytotoxic and Antiviral study of Clevira; JSS academy of higher education and research.
2. OECD (2000) Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No 24.
3. OECD. (2006). Report of the Validation of the Updated Test Guideline 407: Repeat Dose 28-day Oral Toxicity Study in Laboratory Rats. Series on Testing and Assessment No 59, ENV/JM/MONO(2006)26
4. Schlede E., Mischke U., Diener W. and Kayser D. (1994). The International Validation Study of the Acute-Toxic-Class Method (Oral). Arch. Toxicol. 69, 659-670.
5. OECD(2008). Guidance for the testing of chemicals. Repeated Dose 28-Day Oral Toxicity Study in Rodents. 407