

Acute and Repeated Dose Toxicity Study of Clevira Syrup – A Polyherbal Formulation

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This study evaluated the acute and repeated dose toxicity effects of Clevira Syrup Polyherbal formulation (CSPHF), which was prepared from ten different herbs, well known and widely used in traditional medicine for the management of viral infections and other inflammatory disease conditions. Individually these herbs (*Carica papaya*, *Melia azedarach*, *Andrographis paniculata*, *Vetiveria zizanioides*, *Trichosanthes dioica*, *Cyperus rotundus*, *Zingiber officinale*, *Piper nigrum*, *Mollugo Cerviana* and *Tinospora cordifolia*) were completely safe, but the polyherbal formulation effects were not known. Thus, this study was done for the investigation of toxicological profile of CSPHF in Wistar Albino rats. As per OECD (Organisation for Economic Co-operation and Development) guidelines 423 and 407, Acute and Repeated dose toxicity study were proceeded. In the acute toxicity study a single dose of CSPHF (2000mg/kg) was administered orally to female Wistar rats and in repeated dose toxicity study, CSPHF was administered orally in Control group and three different doses (1000, 500 and 250mg/kg body weight) to both male and female wistar albino rats for 28 days. At the end of the study, the animals were euthanized, observed the external and internal morphology (Acute Toxicity) and assessed the effect of CSPHF on histopathological and biochemical parameters (Repeated Dose toxicity study). In acute toxicity study, there were no visual signs of toxicity of CSPHF (2000mg/kg) observed, whereas in Repeated dose toxicity study Ischaemia, inflammation and hematoma of the internal organs were observed at 1000mg/kg dose, but no such toxic features were seen at 500 and 250mg/kg dose of CSPHF. The results of the Acute and Repeated Dose toxicity study could be authenticated in future studies, which will be more useful and evidence based for the management of Viral infections during pandemics.

Keywords: Acute and Repeated Dose Toxicity; Clevira syrup; Polyherbal formulation.

In this modern world, the traditional medicine is more popular, curative, less toxic and with minimal side effects¹. As per World Ethno Botanical reports, 70-80% of global population relies on traditional health care². In India herbal preparations attained wide spread acceptability

with lot of therapeutic value. Most of the medicinal plants in the composition showed pharmacological effects and it had been studied for years. Even today there are new objectives and efficacy profile determined and substantiated with scientific evidences for the benefits of these medicinal plants,

whereas there are limitations for the safety and efficacy of the preparations³.

CSPHF is a combination of ten ingredients, used in traditional medicine for management of viral infection and other disease conditions. The syrup consists of *Carica papaya*, *Melia azedarach*, *Andrographis paniculata*, *Vetiveria zizanioides*, *Trichosanthes dioica*, *Cyperus rotundus*, *Zingiber officinale*, *Piper nigrum*, *Mollugo cerviana* and *Tinospora cordifolia*. These ingredients were found to have anti-inflammatory, anti-pyretic, anti-bacterial, anti-microbial, anti-cancer, antihelmintic, larvicidal, hepatoprotective, anti-diabetic, anti-

obesity and hypolipidemic activity^{4,13}. Apex Laboratories Private Limited, Chennai, has proved that CSPHF has antiviral effects in cell lines with fewer side effects¹⁴.

CLEVIRA Syrup Composition

Each 10ml contain (Aushadh Ghana) extracts derived from medicinal plants of *Carica papaya*, *Melia azedarach*, *Andrographis paniculata*, *Vetiveria zizanioides*, *Trichosanthes dioica*, *Cyperus rotundus*, *Zingiber officinale*, *Piper nigrum*, *Mollugo cerviana* and *Tinospora cordifolia* in mg as described in the Table of Clevira Syrup Composition.

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

MATERIALS AND METHODS

Clevira Syrup was procured from Apex Laboratories private limited, Guindy, Chennai, India.

Ethical Clearance

Adult female wistar Albino rats (weighing 170-200g) were used for acute toxicity study and for Repeated dose toxicity animals of either sex were used after approval by the Institutional Animal Ethics Committee, MGMCRI. (for Acute toxicity study, Ref. 03/IAEC/MGMC/06/2018 – I and Repeated Dose 28 days Toxicity study, Ref. No. 05/IAEC/11/2018 – II).

Animals

The animals were acquired from TANUVAS breeding center, Chennai. The animals were housed in a propylene cage with sterile bedding material, and fed with standard pellet feed and water, which was maintained at a room temperature of $26 \pm 2^\circ\text{C}$ and a relative humidity

45-55% in the Central Animal House, MGMCRI, throughout the study period.

Acute Oral Toxicity

Acute Toxicity testing was done as per the OECD (Organisation for Economic Co-operation and Development) guidelines 423^{15,16}. Female wistar rats were randomly selected and kept in their cages for at least 5 days prior to the initiation of dosing for acclimatization to the laboratory conditions. Animals were divided into 3 groups (N=3) for testing acute oral toxicity. Following overnight fasting, the animals were weighed and the CSPHF (2000mg/kg, PO) was administered and food was withheld for a further 3-4 hours, whereas the control group only received normal saline.

All rats were allowed free access to food and water, and it was observed individually after dosing during the first 30 minutes then periodically during the first 24 hrs with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. In this next 14 days, the animals

were observed once daily for visual observations mortality, physical appearance, behavioural changes and any injury or illness and was recorded with individual records being maintained for each animal. Additional observations were done to see if the animals showed any signs of toxicity (Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention was directed towards observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep 4/14 OECD/OCDE 423 and coma). Individual weights of animals were determined before the test substance was administered and then weekly thereafter. The weight changes were recorded. At the end of the test, animals would be weighed and humanely killed.

All test animals were subjected to gross necropsy and observed for gross pathological changes for each animal. The same procedure was repeated for the second set of test 2 (n=3) using 2000mg/kg dose (Table-1).

Repeated Dose 28days Toxicity study

Acute Toxicity testing was done as per the OECD guidelines 407¹⁷. Following the Acute Oral Toxicity study of Clevira syrup Polyherbal formulation (2000mg/kg), Wistar Albino rats of either sex (n= 5male +5female rats) were divided into four groups (n=5+5) in each group with the allocation as follows: Group I (Control group), Group II (CSPHF 250mg/kg), Group III (CSPHF 500mg/kg) and Group IV (1000mg/kg) Table-2.

Other two groups of rats {n=10 (5+5)} served as Control and Satellite groups based on the 14 days of follow up of the Repeated Dose toxicity study Observations of CSPHF.

The Maximum volume of CSPHF than can be administered was at the dose 1ml/100g body weight. Based on this the dosage of administration of CSPHF was calculated and given orally through oral feeding needle. Following the period of fasting,

Table 1. Acute Toxicity Study of CSPHF

Group	No. of Rats (n)	Dose of CSPHF
Test 1	3 Female Rats	2000mg/kg Body weight
Test 2	3 Female Rats	2000mg/kg Body weight

Table 2. Repeated dose Toxicity Study of CSPHF

Study	Test Drug	Groups	Dosemg/kg	Species/Strain	No. Of Animals and Sex	Total Animals
Repeated Dose Toxicity Study Satellite Group Control Group	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
High Dose of Clevira Syrup			Sterile Water 1ml/100g	Wistar Rats	5 Male rats + 5 Female rats	10
			500mg/kg	Wistar Rats	5 Male rats + 5 Female rats	10

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 administered with the test substance CSPHF at the dose of 250, 500 and 1000mg/kg body weight daily orally for a period of 28 days. 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 chronological days. Individual animal behavioural changes were observed and recorded for each animal at the interval of 7 days as Day 0, Day 7, 14, 21 and 28 respectively. The

animals were monitored for any signs of toxicity of drug from day one of the study and the observations were recorded periodically. During the process of periodical observation at the dose of 1000mg/kg body weight of CSPHF, the animals in this group started to show some of the signs of toxicity (oral ulcers and bleeding from the same, hair fall, conjunctival haemorrhage, spotting of blood at the rectal orifice, skin rashes, urethral orifice with spotting of blood during urination, high coloured urine and black coloured stools) from 15th day onwards along with conjunctival and paw edema, abdomen distension, and hemoptysis. Mortality occurred within 27 days of the study period of all

Table 3. Weight changes in wistarrats baseline, day 7 and day 14 for Acute toxicity study

Groups N=3	Dose of CSPHF	Baseline weight on Day 0 (in gm)	Weight on Day 7 (in gm)	Weight on Day 14 (in gm)
Test 1	2000mg/kg	165+173+169 Avg: 169	170+188+165 Avg: 174.3	167+190+160 Avg: 172.3
Test 2	2000mg/kg	180+179+173 Avg: 177.3	189+190+184 Avg: 187.6	185+189+170 Avg: 181.3

Table 4. Observation on signs of Acute toxicity

Groups	Day 0	Day 7	Day 14
Test 1	Nil	Nil	Nil
Test 2	Nil	Nil	Nil

the animals in Group IV (1000mg/kg of CSPHF). There were no gross pathological changes noted in Group II (250mg/kg of CSPHF) and Group III (500mg/kg of CSPHF). All the orifices, cranial, thoracic cavity, pelvic cavity and abdominal cavity on observation all the internal organs including

Table 5. Weight changes in wistarrats 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	183.4 ± 1.89	180.5 ± 1.17	174.9 ± 2.84	179.3 ± 1.903	183.7 ± 1.202
Test 2 (n=5) Dose of Clevira syrup 500mg/kg	178.5 ± 1.14	181.4 ± 1.91	176.8 ± 1.404	174.8 ± 0.785	171.4 ± 0.66

Table 6. Observation on signs of Repeated dose toxicity

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

the reproductive organs were healthy without any signs of toxicity or necrosis of tissues in Group II and Group III. But on examination of the internal organs and the orifices of the Group IV (1000mg/kg of CSPHF) animals after their mortality, the organs were observed with necrotic tissues with hematoma of the solid organs and ischemia of the colon, with necrosis and gangrenous appearance of the reproductive tissues, etc. Hence the highest safer dose was selected for dosing in the control group and satellite group^{17,18}.

In Satellite group (group V – 500mg/kg CSPHF orally), the animals (n=10) were dosed with the safer highest dose from the repeated dose toxicity (500mg/kg of CSPHF) for a period of 28 days and the control group of animals (n=10) to monitor and compare any specific observations of toxic changes in the animals during the test period.

Weight changes were calculated and recorded weekly. Measurement of food consumption was made at least once weekly. At the end of the study the animals were fasted overnight prior to

necropsy and blood samples for Hematological and Biochemical parameters estimation were collected from retro-orbital puncture after anaesthetising the animals with Intraperitoneal Inj. Thiopentone sodium, following which the samples for histopathological examination were collected after euthanasia of the animals.

Blood Analysis

For haematological analysis different parameters such as Hemoglobin (Hb), Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Platelet Count (PLT), Differential Count (DC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were estimated. Biochemical analysis of serum samples were performed to analyse various parameters such as Serum Creatinine, Aspartate Aminotransferase, Alanine Aminotransferase, Serum Albumin, Globulin, Albumin and Globulin ratio (A/G ratio), total bilirubin, Blood Urea, Blood Glucose and Alkaline Phosphatase.

Table 7. Clevira syrup-Repeated dose toxicity blood analysis parameters
Hematological and Biochemical analysis

Blood analysis	Group 1 Control	Group 2 (250mg/kg)	Group 3 (500mg/kg)
Platelets (10 ³ / μL)	707.8 ± 1.61	768.0 ± 1.49	811.0 ± 4.08
MCHC (g/dL)	33.32 ± 0.18	34.91 ± 0.15	35.09 ± 0.19
MCH (pg)	81.21 ± 0.87	18.54 ± 0.21	19.08 ± 0.15
Hemoglobin (g/dL)	14.43 ± 0.20	14.25 ± 0.10	14.50 ± 1.95
Red blood cells (10 ⁶ / μL)	8.13 ± 0.14	8.11 ± 0.07	8.70 ± 0.149
White Blood cells (10 ³ / μL)	4.49 ± 0.14	4.67 ± 0.24	7.22 ± 0.10
MCV [fL(μm ³)]	45.45 ± 0.19	48.6 ± 0.15	58.61 ± 0.29
% Neutrophils	16.20 ± 1.31	17.80 ± 1.61	17.20 ± 1.619
% Lymphocytes	80.90 ± 1.52	80.0 ± 1.49	81.70 ± 1.76
% Monocytes	2.60 ± 1.74	2.60 ± 1.174	2.20 ± 1.22
% Eosinophils	2.00 ± 0.94	0.10 ± 0.326	0.10 ± 0.316
% Basophils	0.10 ± 0.316	0.10 ± 0.316	0.10 ± 0.316
Total Bilirubin	0.70 ± 0.14	0.09 ± 0.16	0.72 ± 0.10
Alkaline Phosphatase	78.0 ± 1.49	80.20 ± 1.31	81.80 ± 1.61
Aspartate Aminotransferase	89.0 ± 1.49	86.0 ± 1.49	89.0 ± 1.49
Alanine Aminotransferase	38.0 ± 1.49	40.0 ± 1.49	40.0 ± 1.37
Blood Urea	14.66 ± 0.30	15.26 ± 0.11	15.97 ± 0.25
Serum Creatinine	0.34 ± 0.14	0.33 ± 0.14	0.38 ± 0.015
Albumin	3.73 ± 0.12	3.55 ± 0.15	4.14 ± 0.15
Globulin	1.88 ± 0.014	1.77 ± 0.10	1.86 ± 0.15
A/G Ratio	2.10 ± 0.01	2.13 ± 0.12	2.14 ± 0.015

Effect of Repeated dose of CSPHF on Hematological and biochemical Values are expressed as mean ± SEM, P<0.05 when compared to control group CSPHF

Histopathological Examination

After blood collection, the animals were sacrificed by Inj. Thiopentone Sodium i/p and different organs such as Ovary, Testes, Liver, Kidney, Heart, Adrenals and Pancreas were collected from each rat in all groups to observe for any changes in histopathology. The organs were then fixed in 10% neutral buffered formalin for 18hrs at 4 degree Celsius and processed by conventional techniques. Paraffin sections were stained with hematoxylin and eosin, following the standard laboratory procedures. The stained sections were examined under Oil Immersion Microscope under magnification for any change in morphology and for any cellular damage.

RESULTS AND DISCUSSION

Acute Toxicity Study of Clevira Syrup Polyherbal Formulation (Table:3 and 4)

On Observation

The animals in each test group (Test 1 and Test 2) after overnight fasting were observed

for their activity and looked for signs of toxicity. The Orifices and internal organs were carefully examined and observations recorded on Individual basis. During the study none of the animals showed signs of toxicity or had a moribund status. After Inj. Thiopentone Sodium IP the animals were sacrificed and necropsy was done. There was no gross pathological changes noted in any of the groups. Hence from the data obtained after the acute toxicity study the Repeated dose toxicity study of CSPHF was done in the Group of Male and Female Wistar Rats for a period of 28 days.

Repeated Dose 28 days toxicity study of Clevira Syrup Polyherbal formulation in Wistar Albino rats (Table-5 and 6).

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

Table 8. Clevira syrup-Repeated dose toxicity blood analysis parameters_ Hematological and Biochemical analysis: Satellite group

Blood Analysis	Satellite Control	Satellite Group (500mg/kg)
Platelets ($10^3/\mu\text{L}$)	707.8 \pm 1.61	822.20 \pm 1.619
MCHC (g/dL)	33.32 \pm 0.18	34.38 \pm 0.175
MCH (pg)	81.21 \pm 0.87	18.88 \pm 0.15
Hemoglobin (g/dL)	14.43 \pm 0.20	16.40 \pm 0.14
Red blood cells ($10^6/\mu\text{L}$)	8.13 \pm 0.14	8.99 \pm 0.23
White Blood cells ($10^3/\mu\text{L}$)	4.49 \pm 0.14	7.40 \pm 0.149
MCV [fL(μm^3)]	45.45 \pm 0.19	51.15 \pm 16.18
% Neutrophils	16.20 \pm 1.31	20.00 \pm 1.49
% Lymphocytes	80.90 \pm 1.52	79.00 \pm 2.00
% Monocytes	2.60 \pm 1.74	2.20 \pm 1.033
% Eosinophils	2.00 \pm 0.94	2.00 \pm 0.94
% Basophils	0.10 \pm 0.316	0.20 \pm 0.422
Total Bilirubin	0.70 \pm 0.14	0.18 \pm 0.09
Alkaline Phosphatase	78.0 \pm 1.49	80.30 \pm 1.160
Aspartate Aminotransferase	89.0 \pm 1.49	87.20 \pm 1.37
Alanine Aminotransferase	38.0 \pm 1.49	39.10 \pm 0.504
Blood Urea	14.66 \pm 0.30	15.50 \pm 0.14
Serum Creatinine	0.34 \pm 0.14	0.38 \pm 0.015
Albumin	3.73 \pm 0.12	4.30 \pm 0.156
Globulin	1.88 \pm 0.014	1.87 \pm 0.014
A/G Ratio	2.10 \pm 0.01	2.42 \pm 0.307

Effect of Repeated dose -satellite group of CSPHF on hematological and biochemical Values are expressed as mean \pm SEM, $P < 0.05$ when compared to control group CSPHF

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 anesthetized by Inj. Thiopentone sodium I.P Blood samples were collected for Biochemical and Hematological parameters, animals were sacrificed and necropsy was done Table (7 and 8). There were no gross pathological changes of the internal organs 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 In case of utilization of polyherbal formulations we should be clear with some merits and demerits of the combined use of multiple components to demarcate the salient features of each component in the formulation. From the present compound CSPHF during the Acute Toxicity study period the animals in each group were observed for their activity and looked for signs of toxicity from Day 0 to Day 14 once in every 7 days. Every system was carefully examined and recorded on individual basis for each animal.

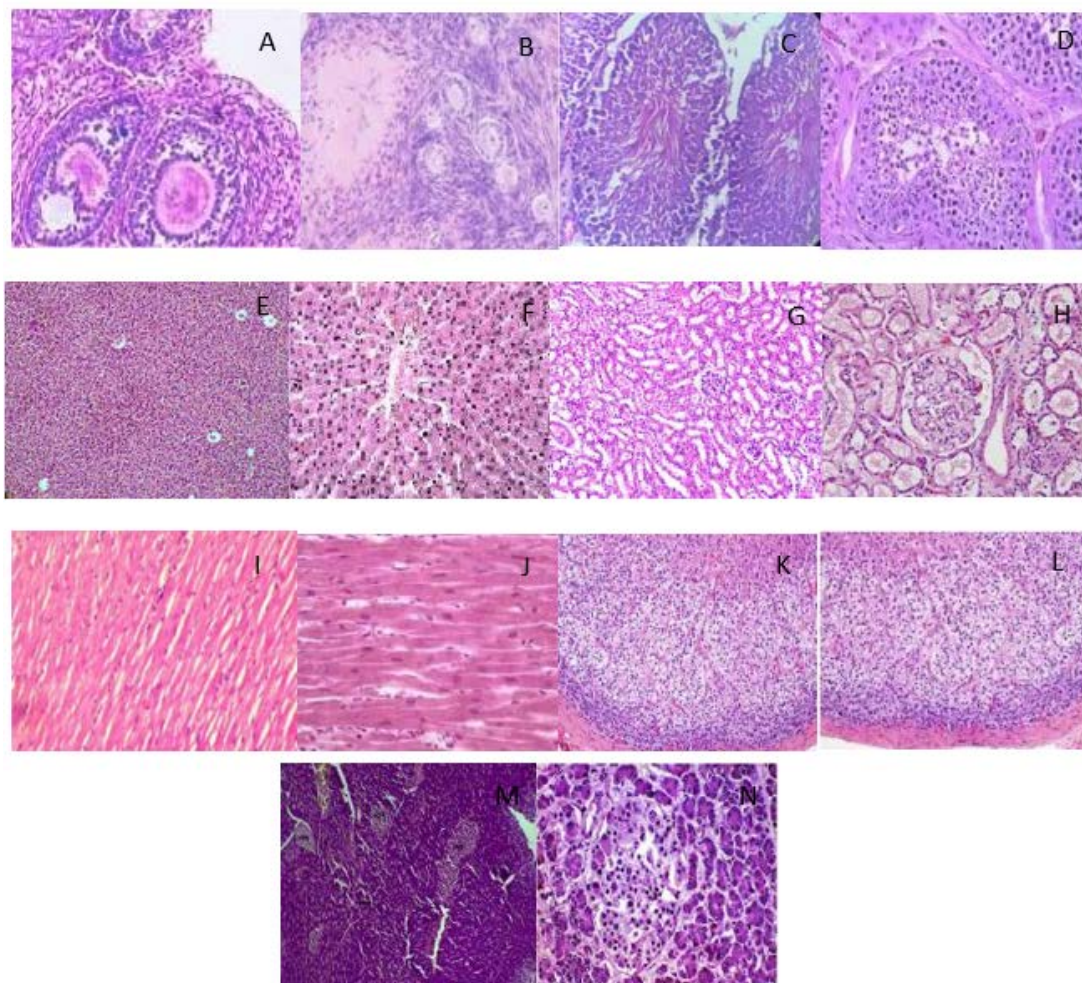


Fig. 1. Histopathological examination of various organs of the rat in repeated dose toxicity study (A,C,E,G,I,K,M) are ovary, testes, liver, kidney, heart, adrenals and pancreas of the control group; (B,D,F,H,J,L,N) ovary, testes liver, kidney, heart, adrenals and pancreas of clevira syrup polyherbal formulation treated group (CSPHF- 500mg/kg)

In this group of animals there were none of the animals which showed signs of toxicity or had a moribund status during the acute toxicity study period.

At the end of the study after gross pathological examination there was no changes noted in any of the groups. Hence from the data obtained the CSPHF was evident to be non-toxic up to single high oral dose of 2000mg/kg body weight without any mortality. Acute toxicity studies showing toxicity signs and mortality for a polyherbal formulation in Wistar Albino rats were meager in the past.^[19,20] Based on this data we further proceeded for Repeated dose toxicity study of CSPHF.

During the repeated dose toxicity study of CSPHF the animals from each group we monitored from day one of the study and the animals were observed for any external features of signs of toxicity. All systems were carefully examined and recorded on individual basis on every seventh day (Day 0, 7, 14, 21 and 28) for 28 days. During the study period at the dose of 250mg/kg and 500mg/kg, none of the animals showed signs of toxicity or ended up with moribund status. But to mention in specific at the dose of 1000mg/kg PO of CSPHF the animals started showing the signs of toxicity (Conjunctival and paw edema, distension of abdomen and hemoptysis, blood stained oral and rectal orifices, increased respiratory rate) from day 15 onwards and then mortality occurred within 27 days, for all the animals in this group with high dose of CSPHF (1000mg/kg).

At the end of the study, animals on examination for gross pathological changes after necropsy the changes noted in Group 1 (250mg/kg, PO, CSPHF) and Group 2 (500mg/kg, PO, CSPHF) were healthy without any signs of inflammation or any other signs of toxicity of the internal organs and normal orifices. But in animals from Group 3 (1000mg/kg, PO, CSPHF), the morbidity and mortality of animals were high even before completion of the study period with inflamed organs on gross pathological examination immediately after the animal mortality.

CONCLUSION

Hence CSPHF (Clevira Syrup Polyherbal formulation) was safe as a single dose at the

maximum level (2000mg/kg, PO) in acute toxicity testing, whereas at repeated dosing of CSPHF the dosage of 500mg/kg and below was found to be safe without any signs of toxicity, morbidity and mortality. We hence plan to continue with chronic toxicity testing of CSPHF to provide more evidence for management with CSPHF without any further signs of toxicity or morbidity on further investigation of the formulation, so it can be useful in chronic viral infections like Hepatitis B, HPV etc, in near future.

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Conflict of interest

There is no conflict of interest.

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There is no Funding Source

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