STUDY TITLE

28-DAYS REPEATED DOSE ORAL TOXICITY STUDY OF AMYRON SYRUP IN WISTAR RAT

STUDY DIRECTOR	TEST FACILITY	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.

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.

Patel

Study Director

Date: 21-12-2020

QUALITY ASSURANCE STATEMENT

The study entitled "**28-Days Repeated Dose oral Toxicity Study of Amyron Syrup in Wistar rats"** has been performed under Good Laboratory Practice Guidelines and in accordance to standard operating procedures and standard protocols. The type of inspection and their dates ,including the phase(s) of study inspected are listed below.The methods, procedures and observations are accurately and completely described, and the reported results accurately and completely reflect the raw data of the study.

Type of the Study	Study-based inspection		
Phase(s) Inspected	Date	Study Director	Management
Study Plan	19-11-2020	Patel	
Critical Phases inspected			
Start of the study	19-11-2020	Patel	
Middle of the study	28-11-2020	Patel	
End of the study	19-12-2020	Patel	
Study Report	21-12-2020	Patel	
Amendment(if any)	No amendment		

The findings of these inspections have been reported to management and the study director on the dates mentioned above

Signature of Mangement:

Date: 21-12-2020

ABBREVIATIONS USED

Abbreviation	Description
g	Gram
kg	Kilogram
%	Percent / percentage
mg	Milligram
No.	Number
Sec	Seconds
ml	Milliliter
µl	Microliter
OECD	Organization for economic cooperation and development

1. STUDY DETAILS

Study Title	:	28-Days Repeated Dose oral Toxicity Study of Amyron Syrup in Wistar rat
Study No.	:	S/RD/TOX/20-008
Test Item	:	AMYRON SYRUP
Sponsor	:	Dr. Anil Kumar Sharma
Study Director	:	Ms. Tulsi Patel
Study Scientists	:	Mr. Satyamanoj M V V
		Ms. Shital
		Dr. Mahesh
		Ms. Jaitra
		Ms. Juli

2. STUDY DETAILS

Study initiation Date	:	19/11/20
Experimental Start Date	:	20/11/20
Experimental Completion Date	:	19/12/20
Study Completion Date	:	21/12/20

3. STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name	Responsibility	Signature
Ms. Tulsi Patel (Study Director)	Overall incharge for the planning, conduct and report preparation of the study	
Mr. Satyamanoj	Assist in conduct of study	

1. SUMMARY

Objective of the present study was to evaluate the 28-days repeated dose oral toxicity of Test Item Amyron Syrup. The study was performed by following OECD test guideline 407.

LIMIT TEST

As per the available information and outcome of acute oral toxicity of Test Item and requirement of sponsor repeated dose Limit Test at the dose level of 20 mL/kg bw/day was performed.

Dose of 20 mL/kg bw/day showed clinical signs of toxicity and mortality in animals on repeated administration.

Hence, Limit Test at 20 mL/kg bw/day was terminated and full study was performed using High, Intermediate, Low and Satellite Dose levels of 20 mL/kg bw/day, 10 mL/kg bw/day, 2 mL/kg bw/day and 20 mL/kg bw/day respectively.

No significant difference in Organ weights i.e. heart, lung, brain kidney spleen, testis (in male animals) and ovary (in female animals) of treatment groups were observed relative to respective male and female control groups after oral treatment for 28 days in Limit Test.

There were few microscopic treatment related findings in animals from treated group when compared with concurrent control group in Skin but no other target organ of toxicity could be identified. In skin Follicular hyperplasia, Increased granular layer in epidermis, Irregular collagen deposition, Langerhan cell proliferation, MNC infiltartion in dermis,PMNC infilteration in dermis, Thickened epidermis with irregular collagen deposition was found which could be treatment related.

FULL STUDY

Treatment groups G-2 (20 mL/kg) and G-5 (20 mL/kg) showed decrease in body weight during the treatment period of 28 days. Female animals of G-2 and G-5 showed comparatively more fall in body weights as compared to Male animals of same respective groups.

G-1 (Control), G-3 (10 mL/kg) and G-4 (2 mL/kg) did not show any significant decrease in body weights during the treatment period of 28 days.

Treatment groups G-2 (20 mL/kg) and G-5 (20 mL/kg) showed gradual decrease in food intake during the treatment period of 28 days. G-1 (Control), G-3 (10 mL/kg) and G-4 (2 mL/kg) did not show any decrease in feed intake during the treatment period of 28 days.

None of the treatment groups showed any significant decrease in water intake during the treatment period of 28 days.

Body weight loss and piloerection was observed in few animals treated with dose level of 20 mL/kg bw/day. No abnormality was observed in G1 (Control), G3 (Intermediate Dose- 10 mL/kg bw/day) and G4 (low Dose- 2 mL/kg bw/day) during the treatment period of 28 days. Reversal in clinical signs was observed in G5 (satellite group- 20 mL/kg bw/day) during the observation period of additional 14 days post treatment.

No mortality was found in male and female animals of of all treated groups respectively.

No abnormality and significant variation was observed in Hematological and Blood Biochemistry profile of any of control/treated groups.

No significant difference in Organ weights i.e. kidney, liver, spleen, testis (in male animals), ovary (in female animals), Heart, lungs and brain of treatment groups of male and female animals were observed relative to respective male and female control groups after oral treatment for 28 days.

There were no microscopic treatment related findings in animals from high dose group when compared with concurrent control group in any of the organs and/or tissues examined and no target organ of toxicity could be identified. Only the skin tissue in male high dose showed changes like Proliferation of granular layer-epidermis(1/5), Thickened epidermis with irregular collagen deposition(1/5), MNC infiltration in epidermis & dermis(1/5) which could be treatment related but did not show any significance. The other observed histopathological findings in control and high dose group animals are considered as either spontaneous or known background findings that are usually observed in laboratory rats of this strain & age

under standard experimental conditions. Moreover, neither their distribution nor their severity had any treatment related association and occurred almost equally in control and high dose groups. Thus histopathology process was not followed in the low dose and mid dose groups.

In light of above mentioned observations, this can be concluded that Test item amyron syrup does not possess any significant oral toxicity following repeated oral administration for 28 days at the high dose level of 20 mL/kg bw/day.

2. OBJECTIVE

To evaluate the 28-days repeated dose oral toxicity study of amyron syrup in Wistar rats. The study was performed by following OECD test guideline 407.

3. SAFETY PRECAUTIONS

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

4. MATERIALS AND METHODS

4.1 TEST ITEM INFORMATION

4.1.1 Test Item-1

Name of the test item	:	AMYRON SYRUP
Appearance	:	Green
Batch No.	:	ARS-2494(D)
Mfg. Date	:	01/2020
Exp. Date	:	12/2022

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	Small Animal Facility - RRCPL
Sex	Male & Female
Source	VAB biosciences, Hyderabad
Number of animals	Limit Test- 20 animals (10 male and 10 female) Full Study- 50 animals (25 male + 25 female)
No. of animals / group	5 animals per group for Limit Test 10 animals (5 male + 5 female) per group for Full Study
Acclimatization	Limit Test- 7 days Full Study- 5 days
Identification of animals	**Pre-randomization-** By tail marking with temporary marker; **Post-randomization-** numbering by ear notching; **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 TG407) for the evaluation of repeated dose oral toxicity study of test item [1].

6.4 ANIMAL HUSBANDRY

Temperature	:	22 ± 3 °C
Relative humidity	:	30 to 70%
Air changes	:	10 to 15 air changes per hour.
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 *ad libitum*. Filtered water was provided *ad libitum*. The diet and water was routinely analyzed for any contaminants that could reasonably by expected to affect the purpose or integrity of the study.

7. EXPERIMENTAL DESIGN AND PROCEDURE

7.1 Principle of Test Method [1]

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

The test substance is applied daily to the skin in graduated doses 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 clinical signs of toxicity and mortality. Animals which die or sacrificed during the test are necropsied and at the conclusion of the test, surviving animals are killed and necropsied.

Signs of toxicity should be recorded as they are observed, including the time of onset, the degree and duration. Cage-side observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern. Measurements should be made of food consumption and animal body weight atleast weekly. Regular observation of the animals is necessary to ensure that animals are not lost from the study due to causes such as cannibalism, autolysis of tissues or misplacement. At the end of the study period all survivors in the non-satellite treatment groups are sacrificed. Moribund animals should be removed and sacrificed when noticed.

7.2 Description of the Test Procedure [1]

Limit Test

20 adult Wistar rats (10 males + 10 females) were taken for the experiment. Animals were acclimatized in Standard Animal House environmental conditions for 6 days prior to the start of experiment. On last day of acclimatization, animals were randomized and allocated to 4 groups of 5 animals each.

G1 & G3 served as Male and Female vehicle control group respectively treated in a similar manner as treatment groups except the administration of Test item. G2 & G4 served as Male and Female Limit Test dose group respectively and treated with 20 mL/kg bw/day of test item administered orally. Allocation of animals has been mentioned as in the table below:

Allocation of Animals

Group	Treatment	Sex	Dose (mL/kg bw /day)	Route of administration	No. of animals / group
G1	Vehicle control*	Male	-	Oral	5
G2	Limit Test Dose	Male	20		5
G3	Vehicle control*	Female	-		5
G4	Limit Test Dose	Female	20		5

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

The animals were treated daily seven days each week for a period of 28 days. Limit Test Dose of 20 mL/kg bw/day was selected (as expected human exposure indicates the need for a higher dose level than 20 mL/kg bw/day).

Limit Test at dose level of 20 mL/kg body weight using the procedure described for this study, produced observable toxic effects / mortality, hence a full study using three dose levels and satellite group was performed.

Full Study

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

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, mid and low dose groups, treated with 20, 10 & 2 mL/kg bw/day of test item respectively.

G5 served as satellite group which was treated in a manner similar with high dose level.

All 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:

Allocation of Animals

Group	Treatment	Dose (mL/kg bw /day)	Route of administration	No. of animals / group
G1	Vehicle control*	-	Oral	5 male + 5 female
G2	High dose group	20		5 male + 5 female
G3	Mid dose group	10		5 male + 5 female
G4	Low dose group	2		5 male + 5 female
G5	Satellite group	20		5 male + 5 female

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

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.

7.3 Preparation of Test items

Calculated amount of Test Item was used undiluted for the oral administration.

7.4 Justification for selection of route of administration

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

8. OBSERVATIONS

8.1 BODY WEIGHT

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

8.2 FOOD/WATER CONSUMPTION

Measurements of food and water consumption was made daily.

8.3 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).

8.4 MORTALITY

All animals were observed twice daily for morbidity/mortality during the entire observation period.

8.5 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 concentration, erythrocyte count, total and differential leucocyte count, platelet count.

8.6 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. Investigations of bilirubin, plasma / serum included glucose, total cholesterol, Triglyceride, HDL, LDL, calcium, chloride, Urea, uric acid, creatinine, total protein and albumin, enzymes indicative of hepatocellular effects (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase).

8.7 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 dosing for 28 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.

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

The following tissues were preserved in the fixation medium for subsequent histopathological examination: Skin, brain, spleen, liver, kidneys, heart and lungs.

8.8 HISTOPATHOLOGY

Full histopathology was carried out on the preserved organs and tissues of all animals of Limit test and Full study.

9. RESULTS

9.1 FULL STUDY

9.1.1 BODY WEIGHT

Treatment groups G-2 (20 mL/kg) and G-5 (20 mL/kg) showed decrease in body weight (statistically insignificant) during the treatment period of 28 days. Female animals of G-2 and G-5 showed comparatively more fall in body weights as compared to Male animals of same respective groups.

G-1 (Control), G-3 (10 mL/kg) and G-4 (2 mL/kg) did not show any significant decrease in body weights during the treatment period of 28 days.

9.1.2 FEED INTAKE

Treatment groups G-2 (20 mL/kg) and G-5 (20 mL/kg) showed gradual decrease in food intake during the treatment period of 28 days.

G-1 (Control), G-3 (10 mL/kg) and G-4 (2 mL/kg) did not show any decrease in feed intake during the treatment period of 28 days.

9.1.3 WATER INTAKE

None of the treatment groups showed any significant decrease in water intake during the treatment period of 28 days.

9.1.4 CLINICAL SIGNS OF TOXICITY

All animal were observed daily during the treatment period for any clinical signs of toxicity.

No abnormality was observed in G1 (Control), G3 (Intermediate Dose- 10 mL/kg bw/day) and G4 (low Dose- 2 mL/kg bw/day) during the treatment period of 28 days.

Reversal in clinical signs was observed in G5 (satellite group- 20 mL/kg bw/day) during the observation period of additional 14 days post treatment.

9.1.5 MORTALITY

No mortality was found in any treated groups.

9.1.6 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.

WBC

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

RBC

The RBC did not show any significant variation and values were found to be within normal limits in all control/treatment groups. Similarly, no significant changes were observed in the red blood cell indices (HCT, MCV, MCH, MCHC etc).

Platelet Count

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

Neutrophil

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

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.

Basophil

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

Reticulocyte

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

9.2.7 CLINICAL BIOCHEMISTRY

SGPT (IU /L)

SGPT level in the treatment groups did not show any significant variation as compared to respective control group but level of SGPT was found high (above normal limits) in all animals (control as well as treatment groups).

SGOT (IU /L)

SGOT level in the treatment groups did not show any significant variation as compared to respective control group but level of SGOT was found high (above normal limits) in all animals (control as well as treatment groups).

Alkaline phosphatase (ALP)

ALP level in the treatment groups did not show any significant variation as compared to respective control group but level of ALP was found high (above normal limits) in all animals (control as well as treatment groups).

Phosphorus

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

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 in the groups treated with 5000 mg/kg bw/day of Test Item (TI) did not show any significant variation as compared to respective control group and values were found to be within normal limits.

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.

Triglyceride

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

Cholesterol

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

Uric acid

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

Chloride

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

Calcium

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

Albumin

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.

HDL, LDL

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

9.2.8 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 drug administration.

No abnormality (on external examination) was found in G1 (Control), G3 (Intermediate Dose- 1000 mg/kg bw/day) and G4 (low Dose- 500 mg/kg bw/day).

Gross internal examination of animals died during treatment period revealed kidneys, lungs and liver.

9.2.9 RELATIVE ORGANS WEIGHT

No significant difference in Organ weights i.e. kidney, liver, spleen, testis (in male animals), ovary (in female animals), Heart, lungs and brain of treatment groups of male and female animals were observed relative to respective male and female control groups after oral treatment for 28 days.

9.2.10 HISTOPATHOLOGY

Histopathological examination was performed on the specified list of tissues including all macroscopically abnormal tissues of control and treated groups animals sacrificed terminally. Five step grading system of minimal (1+), mild (2+), moderate (3+), marked (4+) and severe (5+) were used to rank intensity of the microscopic findings for comparison between the control and high dose group.

There were no microscopic treatment related findings in animals from high dose group when compared with concurrent control group in any of the organs and/or tissues examined and no target organ of toxicity could be identified. Necrotic changes were seen in liver in both sexes of control as well as high dose group. Kidney showed congestion, basophilic renal tubules in both sexes of control and high dose group. Pneumonic lesions including mixed type of cellular infiltration, thickened alveolar wall were noticed in both control as well as high dose group animals. Spleen showed haemosiderosis and extra medullary haematopoiesis. All these lesions

were observed in control and high dose animals with no significant alteration in incidence or severity due to treatment of the test item, therefore these lesions were considered as spontaneous lesions and these could not be correlated as an effect of the test item administration.

10. DISCUSSION & CONCLUSION

Objective of the present study was to evaluate the 28-days repeated dose oral toxicity of Test Item amyron syrup. The study was performed by following OECD test guideline 407.

LIMIT TEST

As per the available information and outcome of acute oral toxicity of Test Item and requirement of sponsor repeated dose Limit Test at the dose level of 5000 mg/kg bw/day was performed.

Dose of 20 mL/kg bw/day showed clinical signs of toxicity and mortality in animals on repeated administration.

Hence, Limit Test at 20 mL/kg bw/day was terminated and full study was performed using High, Intermediate, Low and Satellite Dose levels of 20 mL/kg bw/day, 10 mL/kg bw/day, 2 mL/kg bw/day and 20 mL/kg bw/day respectively.

No significant difference in Organ weights i.e. heart, lung, brain kidney spleen, testis (in male animals) and ovary (in female animals) of treatment groups were observed relative to respective male and female control groups after oral treatment for 28 days.

There were few microscopic treatment related findings in animals from treated group when compared with concurrent control group in Skin but no other target organ of toxicity could be identified. In skin Follicular hyperplasia,Increased granular layer in epidermis, Irregular collagen deposition, Langerhan cell proliferation, MNC infiltartion in dermis, PMNC infilteration in dermis, Thickened epidermis with irregular collagen deposition was found which could be treatment related.

The other histopathological findings in control and treated group animals are considered as either spontaneous or known background findings that are usually observed in laboratory

rats of this strain & age under standard experimental conditions. Moreover, neither their distribution nor their severity had any treatment related association and occurred almost equally in control and treated groups.

FULL STUDY

Treatment groups G-2 (20 mL/kg) and G-5 (20 mL/kg) showed decrease in body weight during the treatment period of 28 days. Female animals of G-2 and G-5 showed comparatively more fall in body weights as compared to Male animals of same respective groups.

G-1 (Control), G-3 (10 mL/kg) and G-4 (2 mL/kg) did not show any significant decrease in body weights during the treatment period of 28 days.

Treatment groups G-2 (20 mL/kg) and G-5 (20 mL/kg) showed gradual decrease in food intake during the treatment period of 28 days. G-1 (Control), G-3 (10 mL/kg) and G-4 (2 mL/kg) did not show any decrease in feed intake during the treatment period of 28 days.

None of the treatment groups showed any significant decrease in water intake during the treatment period of 28 days.

Body weight loss and piloerection was observed in few animals treated with dose level of 20 mL/kg bw/day. No abnormality was observed in G1 (Control), G3 (Intermediate Dose- 10 mL/kg bw/day) and G4 (low Dose- 2 mL/kg bw/day) during the treatment period of 28 days. Reversal in clinical signs was observed in G5 (satellite group- 20 mL/kg bw/day) during the observation period of additional 14 days post treatment.

One mortality was found in male animal of G-5 (satellite group).

No abnormality and significant variation was observed in Hematological and Blood Biochemistry profile of any of control/treated groups.

No significant difference in Organ weights i.e. kidney, liver, spleen, testis (in male animals), ovary (in female animals), Heart, lungs and brain of treatment groups of male and female animals were observed relative to respective male and female control groups after oral treatment for 28 days.

There were no microscopic treatment related findings in animals from high dose group when compared with concurrent control group in any of the organs and/or tissues examined and no target organ of toxicity could be identified. The other observed histopathological findings in control and high dose group animals are considered as either spontaneous or known background findings that are usually observed in laboratory rats of this strain & age under standard experimental conditions. Moreover, neither their distribution nor their severity had any treatment related association and occurred almost equally in control and high dose groups. Thus histopathology process was not followed in the low dose and mid dose groups.

In light of above mentioned observations, this can be concluded that Test item amyron syrup does not possess any oral toxicity following repeated oral administration for 28 days at the high dose level of 20 mL/kg bw/day.

11. REFERENCES

1. OECD guidelines Test Guideline 407

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

One signed copy in original will be archived at test facility

13. ARCHIVES

The study plan, raw data and final report will be archived at the Test Facility for 9 year after completion of the study.