Report of
Pre clinical Safety (Acute) evaluation of Oryzanol

Study No: 02/17

Volume - II

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STUDY JUSTIFICATION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death. Hyperlipidemia is the presence of high levels of cholesterol in the blood and it is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular diseases. Scientists have become increasingly interested in functional foods because of the serious side effects of cholesterol-lowering drugs. Rice is widely consumed globally, and rice bran (RB), a co-product of milled rice, and its oil may have cardiovascular health benefits. Recently, RB has attracted much attention because of its comprehensive nutritional and functional properties. Oryzanol is a class of non-saponifiable lipids of rice bran oil (RBO). More specifically, oryzanol is a group of ferulic acid esters of triterpene alcohol and plant sterols. The quality and quantity of dietary fat is known to play a crucial role of plasma lipid concentration. Hence dietary manipulation is known to play an important role in the management of hyperlipidemia associated obesity and coronary heart disease.

Therefore this project was proposed to have a systematic study of use of oryzanol as cholesterol lowering agent by using standard efficacy models and safety studies. So it becomes mandatory to undertake its efficacy and safety evaluation as per the guidelines of FSSAI and Schedule Y of DCGI. Acute study was conducted in SD Rats.

Signature Page

18.12.2018

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18.12.2018

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1.0 ABBREVIATIONS

Abbreviation	Details
B.Wt.	Body Weight
C	Control
CMC	Carboxy Methyl Cellulose
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
DCGI	Drug Controller General of India
F	Female
FDTRC	Food and Drug Toxicology Research Centre
F.F.A	Free Fatty Acids
GLP	Good Laboratory Practices
gm	Gram
Hr	Hour
HD	Human Dose
IAEC	Institutional Animals Ethics Committee
ICMR	Indian Council of Medical Research
M	Male
PBS	Phosphate Buffered Saline
PC	Prophylactic Concentration
PCT	Pre Clinical Toxicology
NIN	National Institute of Nutrition
SD	Standard Deviation
SE	Standard Error
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
X	Times

2.0 QUALITY ASSURANCE STATEMENT

In accordance with the principles of Good Laboratory Practices (GLP), as a Quality Assurance Officer (QAO) for the **study No. 02/17** entitled **"Pre-Clinical safety Evaluation of Oryzanol".** I visited different divisions involved in the study regularly and inspected the testing facilities and conduct of the investigations as per Standard Operating Procedures (SOPs) at different points of time during the study.

The Institutional Animal Ethics Committee (IAEC) approved to conduct the study (Appendix – III). The report includes the methods and procedures followed by the investigators during the study. The results and inference were given on the basis of the raw data generated during the study.

During the audit of the records of various study investigations, I found that there were no major deviations from the SOPs submitted by the investigators of the Pre–Clinical Toxicology Unit, DTRC, and NIN to the best of my knowledge and belief, which would affect the integrity of the study.

I visited the laboratories on the following dates, audited the records and the findings were reported to the management.

Audit Date	Phase audited	Date reported to Study Director	Date Reported to Management
25.03.2015	Protocol approval	25.03.2015	25.03.2015
14.07.2017	Receipt of test compound	14.07.2017	14.07.2017
31.10.2017	Receipt of animals	31.10.2017	31.10.2017
17.07.2017	Internal Time schedule approval	17.07.2017	31.11.2017
31.10.2017 To 06.11.2017	Acclimatization of animals	31.10.2017 To 06.11.2017	31.10.2017 To 06.11.2017
07.11.2017	Test Compound exposure	07.11.2017	07.11.2017
21.11.2017	Inspection of Euthanization	21.11.2017	21.11.2017

BR. Anapurnna (Q.A.O)

18.12.2018
Date

3. GLP COMPLIANCE STATEMENT

The Study no. **02/17** was performed at PCT (Pre-Clinical Toxicology) the National Institute of Nutrition (NIN), Hyderabad, a public-sector institution established by the Indian Council of Medical Research (ICMR), Government of India. The study was conducted in accordance with the Principles of Good Laboratory Practices (GLP).

DECLARATION

We hereby declare that the work was performed under our supervision in accordance with the described 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 and integrity of the data or which might have had a potential bearing on the validity and reproducibility of this study.

We accept overall responsibility for the technical conduct of the study as well as the interpretation, analysis, documentation and reporting of the results.

Date: 18.12.2018

(Dr. B. Dinesh Kumar)
Study Director & Coordinator – PCT, NIN

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4. STUDY PERSONNEL

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4.2.2 Mr. K. Venkaiah, M.Sc
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4.2.4 Dr. SSYH Qadri, M.V.Sc.
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4.4 Statistics:

Mr. V.Bhaskar, M.Sc. M.C.A

4.5 Archives & Histopathology:

Mrs. K. Sharadha, M.Sc

4.6 Technical Staff (Project):

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2. Mr. V. Nagendra Babu,
3. Ms. T. Lalitha,
4. Mrs. L. Madhavi
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7. Mr. T. Srinivas
8. Ms. H. Anusha Chauhan
9. Ms. B. Shamukhi

5. INTRODUCTION

Rice bran, a coproduct of milled rice, and its oil may have cardiovascular health benefits. Human consumption of rice bran has been limited, primarily because of the rapid onset of rancidity in rice bran, but methods to stabilize rice bran and to extract its oil have been developed. Rice bran contains 10–23% oil and negligible amounts of water-soluble-glucans and larger amounts of insoluble dietary fiber. Rice bran has many food applications in prepared foods, nutraceuticals, and functional foods. Oryzanol is a class of non saponifiable lipids of rice bran oil (RBO). More specifically, oryzanol is a group of ferulic acid esters of triterpene alcohol and plant sterols. The quality and quantity of dietary fat is known to play a crucial role of plasma lipid concentration. Hence dietary manipulation is known to play an important role in the management of hyperlipidemia and obesity and coronary heart disease.

The present study was undertaken to assess the safety of Oryzanol in SD Rats. The report contains the data on safety profile of test material followed by guidelines of FSSAI and Schedule Y of DCGI.

6. SUMMARY

6.1 Title : Acute Toxicity Evaluation of Oryzanol.

6.2 Study Number : 02/17

6.3 Objective : To assess the safety profile of Oryzanol powder in SD Rats by oral route.

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6.6 Test material details

6.6.1 Name of the material : 1. Oryzanol powder

6.6.2 Intended Use : Hypocholesterolemic activity

6.6.3 Appearance : Creamish white colour powder

6.6.4 Composition :

S.No	Composition	Quantity
Oryzanol powder		
1	Natural lipids	5.5 %
2	Oryzanol	92 %
3	Polar lipids	2.5 %

6.6.5 Dosage schedule : Single exposure

6.6.6 Route of Administration : Oral through gavages

6.7 Test system

6.7.1 Test species : Sprague Dawley Rats

6.7.2 Age & weight : 6 – 8 weeks & 180 – 200 grams (±20%)

6.7.3 Study design:

S.No	Test Material	No. of animals	Dosage schedule (oral route)#			Study parameters	Study period*
			Conc.	Volume	Duration		
1	Oryzanol@ powder	10 (5♀ + 5♂)	54mg	2ml	Single dose	Activity and lethality	14 days

* Excluding the conditioning period of 7days.
@ Oryzanol powder dissolved in 0.5% CMC(Carboxy Methyl Cellulose)

6.8 Methodology:

The study has been conducted in Sprague Dawley Rats (5 M + 5 F), aged 6 – 8 weeks old, weighing 180 – 200gm, obtained from NCLAS, NIN, Hyderabad with approval of NCLAS/II-IAEC/2016/BDK/R16F.

6. 8.1 Experimental phase:

The Rats with normal health report have been conditioned for 7 days in the experimental room. This was followed by test compound administration with a concentration of 54mg / 2ml/animal which was 10 times of the intended human dose. Animals were observed daily for 14 days after exposure to the test compound. At the end of the experiment, all animals were euthanized and their organs were collected for gross necropsy. In case of pre-terminal death, an autopsy was conducted to collect the vital organs for histopathological examination.

6.8.2 Study parameters:

The animals were observed for lethality of the test compound. Apart from the routine physical and physiological examinations, body weight gain was recorded bi-weekly till the end of the experiment. Gross necropsy was conducted on all the animals.

6.9 Data retention:

The raw data, observation books, specimens, slides etc and details will be kept confidential at the archiving room for 5 years after the release of final report. The excess test compound provided by the sponsor will be stored under appropriate conditions till the expiry date.

6.10 Statistics

Data was compiled and descriptive statistics was generated using SPSS-19 version software.

6.11 Results:

- No pre-terminal deaths were recorded in any group of the animals.
- No significant effect on body weight gain were recorded
- Clinical signs, behavioral activity were normal.
- Clinical chemistry parameters were in normal range.
- There was no allergenicity symptoms found in the animals.
- There were no significant changes in organ weights.
- No gross necropsy changes were observed in organs collected at the end of experiment in any group of animals.

6.12 Conclusion:

The Oral administration of Oryzanol powder dissolved in 0.5% CMC at a concentration of 270mg/kg rat which was 10 times higher than the Intended human dose, did not show any adverse effect on any of the parameters studied. There was no mortality recorded.

7. Materials and Methods

7.1 Test system

7.1.1 Species and strain : Sprague Dawley Rats

7.1.2 Source : National Centre for Laboratory Animal Sciences
CPCSEA Registration No: 154/1999
ICMR-National Institute of Nutrition,
Hyderabad 500 007, Andhra Pradesh

7.2 Husbandry Practices

7.2.1 Housing & Caging

The animals were housed in a row in standard poly carbonated open cages with top grill having facilities for feed and drinking water in polycarbonate bottles with stainless steel sipper tubes (ad libitum supply).

7.2.2 Environmental conditions

The environmental conditions were maintained at 22 ± 2°C, with 15 -16 air changes per hour and relative humidity of 45 – 55% with a 12 hour light/dark cycle.

7.2.3 Room sanitation

The floor of the experimental room was swept and all work tops and floor were mopped with disinfectant solution every day throughout the study period

7.2.4 Feed and water

The animals were fed on sterile, pellet feed (standard composition, with macro and micronutrients; (Appendix VII); purified water, collected through an activated charcoal filter and exposed to UV rays (Aquaguard online water filter-cum-purifier) was provided to the animals *ad libitum*.

7.3 Acclimatization

All animals were acclimatized for a period of 7 days to initiation of treatment. A thorough physical examination was performed before randomization and only animals free of obvious health abnormalities were used for the study

7.4 Randomization

Randomization ensured that the allocation of treatment to animals was independent of their characteristics and was similar in all the included animals. During randomization, care was taken to ensure that base variables were homogenized and were allotted.

7.5 Animal identification: (SOP NO: 06/PHARM/NIN/CO1/AI/2006/OL)

Each caged animal was identified by cage label showing study title, study number, species and strain, date of important events of the study, regular animal ID, unique ID number and group name (Appendix - VIII).

Group Details	Sex	Unique ID No		No. of animals
		From	To	
Oryzanol powder	M	21531025	21531029	5
	F	21532030	21532034	5

7.6 Test material details: Appendix – IV & V

Test Compounds	:	Oryzanol powder
Date of receipt	:	09.03.2017
Expiry date	:	NA
Concentration	:	54mg/2ml/rat
Name of the Supplier	:	A. P. Organics
Form of dosage	:	Liquid
Route of administration	:	Through Oral gavage
Storage Condition	:	2º C - 8º C

7.7 Test Approvals:

The study has been conducted after obtaining approval of Institutional Animals Ethics Committee (IAEC) (Ref. NCLAS/II-IAEC/2016/BDK/R16F) (Appendix – III).

7.8 Rational for exposure of test material:

i. Oryzanol powder:

Intended human Dose = 300mg/day/adult

Pre clinical dose = Intended dose X conversion factor

= 300mg X 0.018

1XTD = 5.4mg / 200g Rat **/**day

10XTD – 54mg / 200g Rat /day

(or)

270 mg/Kg Rat /day

Therefore 54mg of Oryzanol dissolved in 2 ml of 0.5% CMC and administered to each rat (200gm) /day through oral gavage.

7.9 Experimental Design:

A total of 10 Rats (5 males + 5 females) with normal health report have been conditioned for 7 days in the experimental room to adapt experimental conditions and to get appropriate body weight gain to initiate treatment. This was followed by test compound administration in High Dose [Oryzanol powder (54mg) orally through gavage. The animals were observed for mortality and activity for 14 days. Live phase of animals, cage side observation, physical, physiological and neurological parameters were recorded at regular intervals. This is followed by necropsy and collection of all vital organs viz., brain, thymus, spleen, bone marrow, kidneys, heart, lungs, trachea, thyroid, adrenals, sternum, liver, gastrointestinal tract & testis/ovaries.

7.10 Observations

7.10.1 Functional observation battery

The safety assessment of the test material according to standard international guidelines included monitoring of the following parameters:

7.10.1.1 Live phase of animals (SOP NO: 10/PHARM/NIN/CO1/LOP/2006/OL)

General Behavior

Observations on behavioral abnormalities of the animals viz. active, not active, partially active & hyperactive were recorded and reported bi-weekly

Feed intake

The feed intake was quantified twice a week on a standard electronic balance.

Water intake

The water intake was qualitatively monitored bi-weekly

Body weight

Body weights were recorded bi-weekly at the time of conditioning period, pre and post exposure to the test compound. The weights were recorded using Sartorious electronic balance.

7.10.1.2 Cage-side observations: (SOP NO: 11/PHARM/NIN/CO1/CSA/2006/O2)

The following physiological activities were monitored every day and recorded bi-weekly*:*

Home cage activity

The experiment animals were monitored for their home cage activity (lying on side, resting, alertness, etc.).

Feaces excretion

The quantity, color and consistency of feacal matter were recorded bi-weekly.

Behavior while removing from cage

Behavior of the animal while removing from the cage was recorded: quiet easily removed / runs around in the cage / oriented towards the investigator/aggressive or any vocalization.

7.10.1.3 Physical examination: (SOP NO: 12/PHARM/NIN/CO1/PE/2006/OL)

Physical examinations were made periodically and the following observations were recorded bi-weekly:

Hair coat

The physical appearance of the hair coat (clean/groomed/soiled) was observed bi-weekly.

Lacrimation

The presence of eye secretions if any (slight/moderate/severe) was recorded bi-weekly.

Salivation

The salivation (slight/moderate/excess, etc.) was recorded bi-weekly.

Respiration character and respiratory rate

Respiration character (normal, decreased, increased, shallow, deep, and gasping) was observed.

Eye prominence and eyelid closure

The appearance of the eyes (normal/exophthalmus/endophthalmus) and eyelid closure was also observed.

Biting

Biting character was also recorded

7.10.1.4 Neurological examination: (SOP No: 13/PHARM/NIN/C01/NE/2006/OL)

Neurological activity was monitored biweekly for all animals for the following parameters:

Locomotor activity

The locomotory activity was recorded bi-weekly for all the animals

Rearing activity

The rearing activity includes didn't rear, rear on hind limbs with and without use of tail was observed bi-weekly for all the animals.

Static limb position

The static limb position of the animal was recorded bi-weekly

Tail elevation

The tail elevation includes lifted while walking, occasionally lifted, tail getting injured, tail cut etc. was observed bi-weekly.

Abnormal gait

The abnormal gait viz. spastic, waddling, dragging hind limbs etc. was observed bi-weekly**.**

Ataxic gait

The ataxic gait includes falling frequently; walking inability etc. was observed bi-weekly.

Head position

The position of the head in the animals was recorded bi-weekly during the monitoring of weight gain.

Pinna touch response

The pinna touch response was recorded by touching with a blunt object inside pinna and the response was recorded bi-weekly.

7.10.2 Allergenicity: (SOP NO: 14/PHARM/NIN/CO1/A/2006/OL)

The effect of the test compound on the allergenicity profile was evaluated by following parameters:

Erythema and Alopecia

Skin reactions and hair loss if any were observed every day and recorded bi-weekly.

Eye appearance

Watering and congestion of eyes were observed daily and recorded bi-weekly.

7.10.3 CLINICAL CHEMISTRY:

Blood samples from the rats, fasted for 12 hrs, were drawn on 15th day of test compound exposure from the orbital plexus, collected in heparinized vacutainer tubes and centrifuged at 3000 rpm for 10 minutes to separate the plasma for clinical chemistry analyses. Quality Control sample (Precinorm U) at two levels (level 1 and level 2) supplied by Roche were used to establish the precision and accuracy of the analyses.

Plasma glucose (GLU), Lipid Profile [Cholesterol (CHOL) & Triglyceride (TRI)] were estimated using cobas c 311 analyzer supplied by Roche at the Food and Drug Toxicology Research Centre, National Institute of Nutrition, Indian Council of Medical Research. All analytical kits were purchased from Roche.

7. 10. 4 Gross necropsy

7.10.4.1 Gross changes:

After the experimental period, the rats were fasted overnight (water was provided) and euthanized by using CO_2 chamber. Gross necropsy was conducted to all animals surviving till the end of the experiment. If any animal died during the study, its organs were subjected to histopathological evaluation.

7.10.4.2 Organ weights:

After detailed gross necropsy examination, the following organs were collected from each animal and the weights were recorded using a top loading electronic weighing machine: Liver, Spleen, Kidney, Lungs, Heart, Brain and Testes.

7.11 Archiving:

All sensitive data, documentation, records, specimens, slides, protocols and final reports generated as a consequence of this study will be kept confidential, inventoried, and archived by the Archiving Officer in the Archives Room of the main building of NIN. The retention duration of these records and the excess test compound provided by the sponsor will be kept under appropriate storage conditions till 5 years after the release of the final report.

7.12 Statistical analysis:

The Department of Statistics was responsible for the statistical analysis of the study data, and was involved in the entire study right from the planning stage.

7.12.1 Sample size determination:

The sample size required for the study was determined as per the protocol of the regulatory authorities.

7.12.2 Study design:

The study design takes an ‘a priori’ rationale for the target difference between the treatments and the control. This was designed to detect the difference, and also the power to detect such a difference taking into account all the available relevant information in the judgment of statistical differences observed for clinical differences. Proper measures were always taken to avoid bias particularly by applying randomization methods, local control methods and blinding of the study.

7.12.3 Blinding:

Utmost care was taken to maximize the degree of blindness. The investigators or the analysts who were concerned with this study did not know which treatment was given to which group. This was maintained until the completion of the project, when the code was broken for the analysis.

7.13 STUDY RESULTS

7.13.1 Pre terminal Deaths:

There were no pre–terminal deaths throughout experimental period. [Table-I].

7.13.2 Functional Observation Battery:

7.13.2.1 Live Phase of Animals:

General Behavior

All the animals were active before and after the exposure to test compound.

Water intake

The water intake was found to be adequate in all animals.

Feed intake

The Feed intake was found to be adequate in all animals.

Body weight

No abnormal findings with reference to gain in body weights during the experimental phase [Table – II].

7.13.2.2 Cage Side Observations:

Cage side observations were conducted twice in a week. The following observations were made.

Home cage activity

The animals were found sitting. No animal was found to be circling purposelessly in the cage during the experiment. There was no abnormal activity.

Faeces excretion, consistency & color

There were no abnormalities in the faeces excretion, consistency & color during the experimental period.

Urine Output

There were no abnormalities in the output during the experimental period.

Urine color

The color of the urine in all the animals was found to be normal.

Behaviour while removing from cage

100% of the animals were quiet easily removed from the cages. None of the animals showed vocalization and aggressive behavior.

7.13.2.3 Physical Examination:

Physical examination was conducted twice in a week. The following observations were made

Hair coat

Clean groomed hair coat was observed in all the animals exposed to test compound.

Piloerection

No piloerection was observed in animals exposed to test compound.

Lacrimation

No excessive lacrimation was observed during the experimental period in all animals.

Salivation

No excessive salivation was observed during the experimental period in all animals.

Respiration character and rate

There were no significant abnormalities in respiration character and rate in all animals during the experimental phase.

Eye prominence & Eyelid (s) closure

The eyes were found to be normal and the eyelids were open in all animals.

Biting behavior

There was no aggressiveness and biting character observed.

Convulsions

There were no convulsions recorded immediately after the administration of test compound and during post exposure in any of the animals.

Tremors

There were no tremors in the animals before and after exposure to the test compound.

7.13.2.4 Neurological Examination:

The neurological observations are indicated in

Locomotor activity

The locomotor activity was found to be normal in all animals.

Rearing activity

None of the animals was found to be rearing on hind limbs with use of tail.

Tail elevation, Head position

All the animals lifted tail while walking during the observation period.

Static limb position, abnormal gait

There were no abnormal limb positions, gait in animals.

Head position

Head position of all animals was normal without tilt.

Pinna touch response

All animals showed normal response to pinna touch.

7.13.2.5 Clinical Chemistry:

The clinical chemistry parameters viz., blood glucose, cholesterol and Triglyceride levels were found to be in normal range in all groups of animals when compared to control [Table-III].

7.13.3 Gross Necropsy:

7.13.3.1 Gross changes:

No gross changes were observed in any of the organs examined during necropsy [Table-V].

7.13.3.2 Organ weights

No changes in organ weights were observed in the animals [Table-IV].

8.0 CONCLUSION:

The Oryzanol powder (test material) administered orally at a concentration of 270mg/kg rat which was 10 times higher than the Intended human dose did not show any adverse effect on any of the parameters studied. There was no mortality recorded

9.0 TABLES

TABLE – I

MORTALITY OF RATS

S. No	Day of observation	Oryzanol powder	
		M	F
1	1	0/5	0/5
2	2	0/5	0/5
3	3	0/5	0/5
4	4	0/5	0/5
5	5	0/5	0/5

Number of animals that died / total number of animals.

TABLE – II

BODY WEIGHTS (gm)

	Observation day				
	Baseline	4th day of exposure	7th day of exposure	11th day of exposure	14th day of exposure
Sex pooled	187.06 ± 17.068 (10)	191.22 ± 18.685 (10)	199.87 ± 19.939 (10)	208.22 ± 22.309 (10)	215.46 ± 21.461 (10)
Male	198.30 ± 14.354 (5)	204.78 ± 16.125 (5)	213.94 ± 17.728 (5)	224.82 ± 18.913 (5)	231.82 ± 17.220 (5)
Female	175.82 ± 11.557 (5)	177.66 ± 8.114 (5)	185.80 ± 9.237 (5)	191.62 ± 8.557 (5)	199.10 ± 8.404 (5)

Values are expressed as Mean ± Standard Deviation ( ) No of animals

Biweekly values are provided in statistical analysis

TABLE – III

BIO CHEMISTRY

Groups Exposure	Oryzanol powder group		
	Sex pooled	Male	Female
Parameters			
Glucose (mg/dl)	86.10 ± 6.064 (10)	89.40 ± 6.465 (5)	82.80 ± 3.701 (5)
TP (g/dl)	6.61 ± 0.341 (10)	6.59 ± 0.419 (5)	6.62 ± 0.294 (5)
Lipid Profile			
Cholesterol (mg/dl)	75.03 ± 15.951 (10)	61.96 ± 10.805 (5)	88.10 ± 5.356 (5)
TRI (mg/dl)	39.90 ± 11.142 (10)	43.52 ± 10.664 (5)	36.28 ± 11.525 (5)
HDL (mg/dl)	57.08 ± 11.486 (10)	47.96 ± 5.058 (5)	66.20 ± 7.957 (5)
LDL (mg/dl)	11.37 ± 1.366 (10)	11.13 ± 1.759 (5)	11.60 ± 0.986 (5)
Liver Function Test			
ALT (U/L)	48.98 ± 17.196 (10)	61.20 ± 13.280 (5)	36.76 ± 10.752 (5)
AST (U/L)	112.93 ± 30.751 (10)	119.26 ± 33.482 (5)	106.60 ± 30.107 (5)
ALP (U/L)	168.90 ± 45.069 (10)	199.60 ± 40.265 (5)	138.20 ± 24.345 (5)

Values are expressed as Mean ± Standard Deviation ( ) No of animals
Appendix – VI (A) for normal range values

TABLE – IV

ORGAN WEIGHTS (g/100g Bwt)

Organs	Brain	Heart	Lungs	Liver	Spleen	Kidney	Testis
Sex pooled	0.70 ± 0.145 (10)	0.39 ± 0.078 (10)	0.18 ± 0.047 (10)	3.12 ± 0.230 (10)	0.25 ± 0.024 (10)	0.72 ± 0.175 (10)	1.76 ± 0.284 (10)
Male	0.62 ± 0.166 (5)	0.36 ± 0.097 (5)	0.16 ± 0.045 (5)	2.99 ± 0.134 (5)	0.24 ± 0.020 (5)	0.81 ± 0.060 (5)	1.76 ± 0.284 (5)
Female	0.79 ± 0.030 (5)	0.42 ± 0.047 (5)	0.21 ± 0.032 (5)	3.24 ± 0.250 (5)	0.26 ± 0.024 (5)	0.63 ± 0.209 (5)	-

Values are expressed as Mean ± Standard Deviation

TABLE – V

NECROPSY FINDINGS – SEX POOLED

DOSAGE	Oryzanol powder	
Sex	**M**	**F**
Number of Animals	5	5
Mortality during treatment	0	0
Moribund and sacrificed	0	0
Finally sacrificed	5	5
Examined for gross pathology	5	5
Gross pathology	0	0
No visceral organ pathology	5	5

10. APPENDICES

APPENDIX – I

LETTER FROM SPONSOR

Ricela®

A.P. ORGANICS LIMITED
Saron Road, DHURI - 148 024 (Pb.) India
Ph.: 01675- 220700, 221100, 225862, Fax. 01675-228204
(An ISO 9001:2008 and HACCP Certified Company)

The Director,

National Institute of Nutrition ,

Hyderabad.

Dated : 19-07-2014.

Kind Attention.

Dr. B. Dinesh Kumar,

Deputy Director.

Sub: Regarding conduction of Efficacy, Safety and Clinical trials for Oryzanol extracted from Rice Bran oil.

Dear Sir,

The company A.P.Organics Limited is part of Ricela Health Foods Limited (a national award winning company and India's largest processor of refined rice bran oil) . The chairman of the company Dr. A. R. Sharma is currently the global President of International Rice Bran Oil Promotion Council. Apart from producing good quality rice bran oil other vision of the company is to add value to the byproducts generated during the processing of rice bran and oil.

In this regard with guidance of IICT , Hyderabad, the company has developed Oryzanol from refined rice bran oil (with 75-80% purity, a natural antioxidant efficient for cholesterol management) which has to tested for efficacy, safety and clinical studies. The project is in collaboration with BIRAC (earlier with DBT). As NIN is the premier institute of India in this field , the company would like to associate with NIN for undertaking the trials.

Hereby the company request NIN to give approval and to design a protocol for conduction of Pre Clinical- Efficacy and Safety trials followed by Clinical studies.

The company will look forward for your consent and valuable guidance.

Regards,

Varun Goyal,Head (R&D),

A.P.Organics Limited,

Dhuri-148024,

Dist. Sangrur, Punjab.

Tel: 01675-228900/ Mob: +91-9815162850.

nlocking the health secrets, hidden in Rice Bran

E-mail: apsolvex@ricela.com | visit us at: www.ricela.com | Regd. Office : IInd Floor, Jewel Plaza, College Road, Civil Lines, Opp. Sita Ram's, Ludhiana, Punjab.

APPENDIX – II

CONSENT LETTER

Website : www.ninindia.org
Fax : 91-40-27019074
E-mail : dirnin_hyd@yahoo.co.in

Phone : Off. 91-40-27018083
Res. 91-40-27171307

राष्ट्रीय पोषण संस्थान
NATIONAL INSTITUTE OF NUTRITION
भारतीय आयुर्विज्ञान अनुसंधान परिषद
Indian Council of Medical Research
जामे उस्मानिया, हैदराबाद - ५०० ००७
Jamai-Osmania P.O. Hyderabad - 500 007, AP

डॉ. कल्पगम पोलासा, पि हेच.डि, एम् बि ए
वैज्ञानिक 'एफ', एवं प्रमुख, एफ .डी.टी.आर.सी
प्रभारी निदेशक

Dr. Kalpagam Polasa, Ph.D, MBA
Scientist 'F' & Head, FDTRC
Director Incharge

Ref: D/NIN/PCT/09/14/
15th September, 2014

Dear Mr. Varun Goyal

Thank you for the visit to our Institute and propose to undertake the collaborative project titled "**Pre clinical Efficacy and Safety evaluation of Oryzanol", A.P.Organics Ltd., Dhuri, Punjab** as Public Private Partnership program at our Centre.

The project will be undertaken with funding source from BIPP of DBT - **Biotechnology Industry Research Assistance Council (BIRAC).** The experimental work will be carried out after obtaining approvals from Institutional Animal Ethical Committee (IAEC), signing MOU and release of grants. I am nominating my colleague Dr. B. Dinesh Kumar, Scientist 'E' & Group Leader - PCT as Study Director to coordinate all activities related to this program. Please find enclosed the minutes of meeting held on 8th September, 2014 at NIN.

The detailed protocol along with budget will be released after receiving the payment of Rs. 50,000/-. The mode of payment should be through cheque or Demand Draft payable in the name of Director, National Institute of Nutrition at any Nationalised bank in Hyderabad or through E-Transfer with information to us (enclosure - I). If you undertake the experimental investigation with us, then the protocol charges will be adjusted at the end of study program.

Thanking you

Yours sincerely

(Kalpagam Polasa)

To
Mr. Varun Goyal
A.P.Organics Ltd.,
Saron Road,
Dhuri(Punjab)
Phone No: 01675-228900

C.C to
1. Dr R. B. N. Prasad, Chief Scientist & Head, Centre for Lipid Research, CSIR-IICT Hyderabad
2. Dr. B. Dinesh Kumar, Scientist 'E' & Group Leader–PCT, NIN (ICMR), Hyderabad

APPENDIX – III

IAEC APPROVAL

Email: nclas123@yahoo.com
Website: www.ninindia.org

Telephone 91-40-27197201/ 205 /207
Fax 91-40-27003317
Mobile 91-9849178671

राष्ट्रीय प्रयोगशाला पशु विज्ञान केन्द्र
NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES
(Regd.No. 154/GO/RBi/SL/99/CPCSEA)
NATIONAL INSTITUTE OF NUTRITION
(Indian Council of Medical Research)
Jamai-Osmania PO, Hyderabad - 500 007, Telangana State

Dr. P. SURESH
Director In-Charge, NARF-BR
Scientist 'G', Head NCLAS &
Member Secretary, IAEC

NCLAS/IAEC/01/2017/R07
Date: 10th July, 2017

To
Dr. B. Dinesh Kumar
Principal Investigator
Scientist 'F', Deputy Director (Sr. Gr)
HOD Drug Toxicology Division
NIN, Hyderabad

Dear Dr. B. Dinesh Kumar,

This is to inform you that the Institutional Animal Ethics Committee has **approved for renewal** of your project entitled **"Pre-Clinical efficacy and safety evaluation of Oryzanal"** The study number is **:** R07/2017/P3F/III-IAEC/2014/BDK/SD-16M+16F/SD-20M+20F/G. Pig/36M, please refer the same number when making correspondence related to this project.

The necessary forms for indenting animals are available in NCLAS office

Yours faithfully

(Dr. P. Suresh)

APPENDIX – IV

CERTIFICATE OF ANALYSIS

सीएसआईआर-भारतीय रासायनिक प्रौद्योगिकी संस्थान
हैदराबाद - 500 007.भारत
CSIR - Indian Institute of Chemical Technology
Hyderabad - 500 007. INDIA
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद)
(COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH)

Ref: IICT\LSTD\IND\63

दिनांक / Dated January 18, 2017

Dr. B. V. S. K. Rao
Principal Technical Officer
Lipid Science and Technology Division

Dr. B. Dinesh Kumar
Scientist 'E' (Deputy Director) &
Co-ordinator – PCT
Food and Drug Toxicology Research Centre (FDTRC)
National Institute of Nutrition (NIN)
Indian Council of Medical Research (ICMR)
Jamia-Osmania P.O., Hyderabad – 500007

Dear Dr. Dinesh Kumar,

I am herewith providing the composition of the Oryzanol sample received from M/s A. P. Organics Ltd., Dhuri

Composition of Oryzanol sample received on 10-01-2017 from M/s A.P. Organics Ltd

Component	Wt. %
Neutral Lipids (like triglycerides, steryl esters, hydrocarbons)	5.5
Oryzanol	92
Polar lipids (like phytosterols and partial glycerides)	2.5

With regards

Yours sincerely,

[B.V.S.K. Rao]
Tel (O): 91-40-27191848
Telefax: 91-40-27193370
Email: raobvsk@gmail.com
bhamidipati@iict.res.in

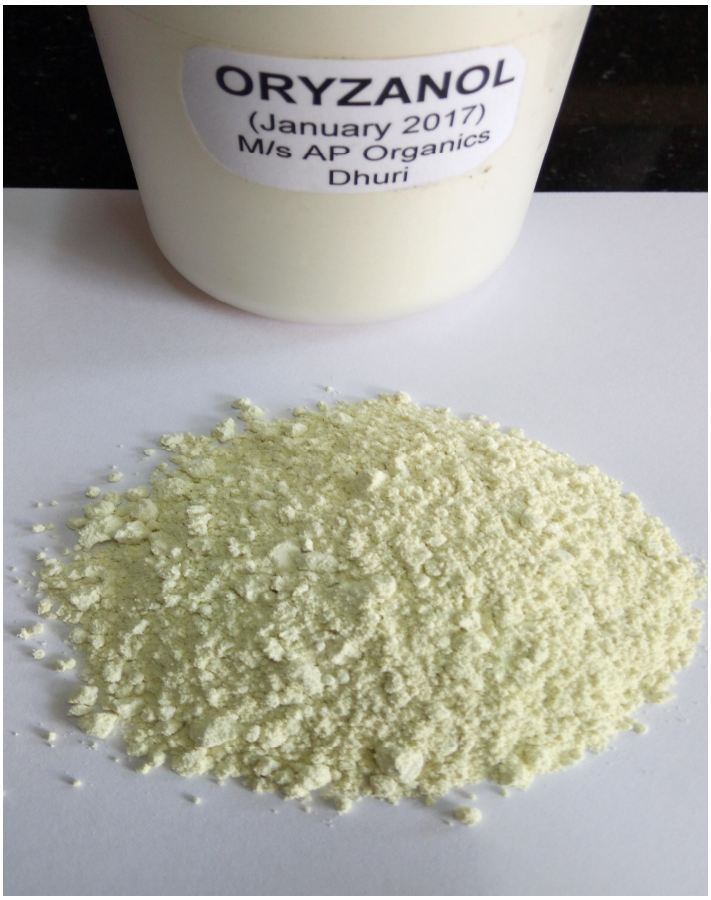
Copy to:

Sri Varun Goyal
A.P. Organics Ltd.,
Saron Road
Dhuri – 148 024
Punjab State

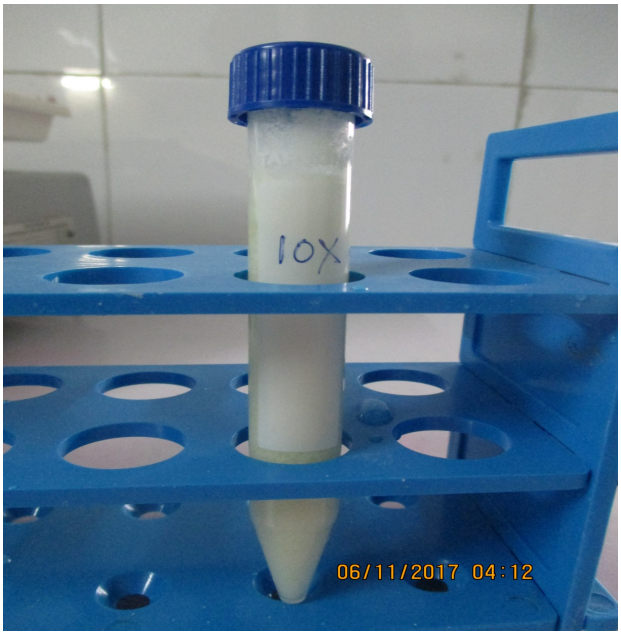
TARNAKA, UPPAL ROAD, HYDERABAD - 500 007. Telangana State, INDIA
दूरभाष / TELEPHONE: 27160123 (18 लाइन / 18 Lines)
www.iictindia.org निदेशक / Director : Fax : 91-40-27160387 व.प्र.नि / COA : Fax : 91-40-27193198

APPENDIX – V

TEST MATERIAL PHOTOGRAPHS



PREPARED TEST MATERIAL



APPENDIX – VI

(Standard Operating procedure)

Standard Operating procedure for Preparation and administration of test material in Rat

1. As per the protocol, calculate the required amount of test material to be given to different group of animals.
2. Obtain the required amount of test material from the custodian after filling the 'Form– B'.
3. Weigh 5gms CMC and mix with 400 ml diluents (HPLC grade water) and stir it well on magnetic stirrer and kept it for overnight and the next day make upto 1000 ml with diluents and label it as 0.5% CMC,
4. Weigh the required amount of test compound according to the animal body weight and mix with 15ml of diluent (0.5%CMC solution) to test compound and Sonicate it for 10-15 minutes and label it separately with the group names.
5. Remove the rat from the cage and restrain it carefully.
6. Draw the solution into a 2-ml syringe with a 18-gauge oral gavage needle, and administer the TC by carefully inserting the needle into the mouth of the rat and proceeding via the oesophagus.
7. After administration, monitor the animal for some time.
8. Replace the rat in its cage.
9. The above procedure should be followed for all the animals.

APPENDIX – VII

DIET COMPOSITION FOR RAT

S.No.	Materials	Percentage
1	Wheat flour	22.5 %
2	Roasted Bengal gram flour	60 %
3	Skim Milk Powder	5 %
4	Casein	4 %
5	Refined Oil	4 %
6	Salt Mixture with starch	4 %
7	Vitamin & Choline mixture with starch	0.5 %

Scale of diet per mouse:

Water and diet were provided *ad libitum*

SALT MIXTURE COMPOSITION FOR RAT

S.No.	Minerals	Per 100 Kg diet (g)
1	Dicalcium Phosphate (Ca_2HPO_4)	1250.00
2	Calcium carbonate ($CaCO_3$)	555.00
3	Sodium Chloride (NaCl)	300.00
4	Magnesium sulphate (Mg SO_4 $7H_20$)	229.00
5	Ferrous Sulphate (Fe SO_4 $7H_20$)	108.00
6	Manganese sulphate ($MnSO_4$ H_20)	16.04
7	Potassium Iodide (KI)	1.00
8	Zinc sulphate ($ZnS0_4$ $7H_20$)	2.192
9	Copper sulphate ($CuSO_45H_20$)	1.908
10	Cobalt Chloride ($CoCl_26H_20$)	0.012

All minerals together : 2463.15

Starch : 1536.85

=======

4000–00 g

======

i.e., 4.0 kg for 100 kg of diet

APPENDIX – VII

DIET COMPOSITION - VITAMIN MIXTURE COMPOSITION (Contd...)

S.No.	Minerals	Per 100 Kg diet (g)
1	(dl) – α Tocopherol Acetate 50% Dry Powder	12.0 g
2	Menadione (K)	0.15 g
3	Thiamine (B_1)	1.2 g
4	Riboflavin (B_2)	0.5 g
5	Pyridoxine (B_6)	0.6 g
6	Niacin	1.0 g
7	Pantothenic Acid (Calcium Salt)	1.2 g
8	Cyanocobalamine	0.5 mg
9	Folic Acid	0.1 g
10	Para amino Benzoic Acid (PABA)	10.0 g
11	Biotin	40.0 mg
12	Inositol	10.0 g
13	Choline Chloride	100.0 g

Total vitamins put together : 177.250 g

Starch add to make up : 500.000 g

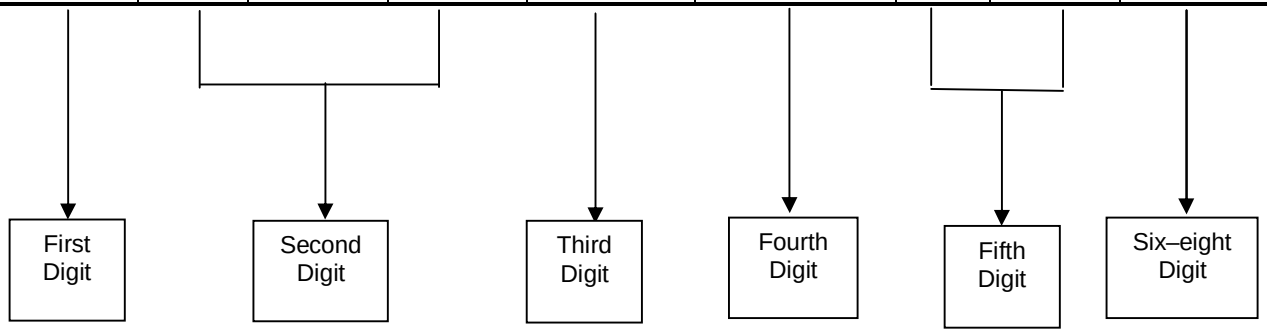
i.e. 500 g of vitamin mixture is used for every 100 kg of the diet prepared.

NIN Standard Pellet Diet

APPENDIX – VIII

CODE SEQUENCE PROCEDURE

STUDY NO	STUDY CATEGORY			SPECIES*	TEST GROUPS	SEX		SERIAL NO. (Animal No.)
	Acute	Sub acute	Chronic			Male	Female	
1 – 9	1	2	3	4 (1 – 6)	1–6	1	2	001 – 999



*

Species name	Code
Mice	1
Monkey	2
New Zealand mice	3
Guinea pigs	4
Rat	5
Rabbit	6
Hamsters	7

APPENDIX – IX

REFERENCES

1. Schedule 'Y' of Drugs & Cosmetics Act, 1940 and Rules, 1945.
2. Conversion factor to rats [Paget.G.E. & Barnes.J.M. (1964) Evaluation of Drug Activities: Pharmocometrics Ed. Laurence.D.R & Bocharach.A.L., Vol.1. Academic Press, New York].
3. Revised RDA for Indians 2010, National Institute of Nutrition

11. STATISTICAL ANALYSIS

Acute Oral Safety of Oryzanol in SD Rats – Body weights

Report

Sex		Baseline	4th day of exposure	7th day of exposure	11th day of exposure	14th day of exposure
Male	N	5	5	5	5	5
	Mean	198.30	204.78	213.94	224.82	231.82
	Std. Deviation	14.354	16.125	17.728	18.913	17.220
	Minimum	176.6	183.8	189.6	201.4	206.3
	Maximum	215.7	226.5	236.7	251.4	252.2
Female	N	5	5	5	5	5
	Mean	175.82	177.66	185.80	191.62	199.10
	Std. Deviation	11.557	8.114	9.237	8.557	8.404
	Minimum	160.3	167.4	178.3	181.7	189.5
	Maximum	188.2	186.5	199.4	200.5	206.4
Total	N	10	10	10	10	10
	Mean	187.06	191.22	199.87	208.22	215.46
	Std. Deviation	17.068	18.685	19.939	22.309	21.461
	Minimum	160.3	167.4	178.3	181.7	189.5
	Maximum	215.7	226.5	236.7	251.4	252.2

Acute Oral Safety of Oryzanol in SD Rats – Bio chemistry

Report

Sex		GLU	HDL	TP	CHOL	TRIGL	ASTL	ALTL	ALP	LDLC3
Male	N	5	5	5	5	5	5	5	5	5
	Mean	89.40	47.96	6.59	61.96	43.52	119.26	61.20	199.60	11.13
	Std. Deviation	6.465	5.058	0.419	10.805	10.664	33.482	13.280	40.265	1.759
	Minimum	84.0	42.7	6.25	46.3	28.1	84.6	49.1	155.0	9.28
	Maximum	100.0	54.6	7.21	73.4	56.3	172.0	79.8	264.0	13.53
Female	N	5	5	5	5	5	5	5	5	5
	Mean	82.80	66.20	6.62	88.10	36.28	106.60	36.76	138.20	11.60
	Std. Deviation	3.701	7.957	0.294	5.356	11.525	30.107	10.752	24.345	0.986
	Minimum	78.0	53.9	6.13	82.8	20.1	84.7	21.3	111.0	10.44
	Maximum	87.0	75.2	6.91	95.7	48.3	159.5	51.2	165.0	13.14
Total	N	10	10	10	10	10	10	10	10	10
	Mean	86.10	57.08	6.61	75.03	39.90	112.93	48.98	168.90	11.37
	Std. Deviation	6.064	11.486	0.341	15.951	11.142	30.751	17.196	45.069	1.366
	Minimum	78.0	42.7	6.13	46.3	20.1	84.6	21.3	111.0	9.28
	Maximum	100.0	75.2	7.21	95.7	56.3	172.0	79.8	264.0	13.53

Acute Oral Safety of Oryzanol in SD Rats – Organ weights

Report

Sex		Brain	Heart	Lungs	Liver	Spleen	Kidney	Testis
Male	N	5	5	5	5	5	5	5
	Mean	.62	.36	.16	2.99	.24	.81	1.76
	Std. Deviation	.166	.097	.045	.134	.020	.060	.284
	Minimum	.326	.196	.085	2.891	.210	.744	1.301
	Maximum	.735	.436	.211	3.214	.257	.900	1.987
Female	N	5	5	5	5	5	5	
	Mean	.79	.42	.21	3.24	.26	.63	
	Std. Deviation	.030	.047	.032	.250	.024	.209	
	Minimum	.761	.354	.172	3.052	.237	.259	
	Maximum	.839	.472	.248	3.631	.291	.771	
Total	N	10	10	10	10	10	10	5
	Mean	.70	.39	.18	3.12	.25	.72	1.76
	Std. Deviation	.145	.078	.047	.230	.024	.175	.284
	Minimum	.326	.196	.085	2.891	.210	.259	1.301
	Maximum	.839	.472	.248	3.631	.291	.900	1.987