1.0 TITLE PAGE

CLINICAL STUDY REPORT

A double blinded, balanced, randomized, two treatment, two period, preliminary microbiome study of Analemma Water and assess the NAD+ assay from whole blood pre and post treatment of Analemma Water in normal, healthy, adult, human subjects.

<table>
<tr><td>Study code</td><td colspan="4">BE/23/378</td></tr>
<tr><td rowspan="2">Investigational product</td><td colspan="4">Test Product: Analemma Water</td></tr>
<tr><td colspan="4">Reference Product: Placebo drinking water tube</td></tr>
<tr><td>Study design</td><td colspan="4">A double blinded*, balanced, randomized, two treatment, two period, preliminary microbiome study and assess the NAD+ assay.

*Person involved in product handling, dispensing and dosing were not involved in particular assessment which causes biasness. e.g., AE monitoring and management. Subject participating in the study were also blinded about the treatment (Test/ Placebo) to be administered.</td></tr>
<tr><td rowspan="6">Study dates (DD/MM/YY)</td><td colspan="2">Phase</td><td>Initiation</td><td>Completion</td></tr>
<tr><td colspan="2">Screening</td><td>05/06/24</td><td>08/06/24</td></tr>
<tr><td colspan="2">Clinical</td><td>08/06/24</td><td>13/09/24</td></tr>
<tr><td rowspan="2">Analysis</td><td>Day 00</td><td colspan="2">09/06/24</td></tr>
<tr><td>Day 96</td><td colspan="2">13/09/24</td></tr>
<tr><td colspan="2">Statistics</td><td>09/10/24</td><td>09/10/24</td></tr>
<tr><td>Clinical Investigator</td><td colspan="4">Dr. Yashvant Khaire
Raptim Research Pvt. Ltd., Navi Mumbai - 400 710, India.</td></tr>
<tr><td>CRO</td><td colspan="2">Screening and Clinical Facility and Statistical Facility (NAD study):
Raptim Research Pvt. Ltd.,
Clinical Unit (PAP-213, A-226);
Biostatistical Unit (A-242);
T.T.C., Industrial Area, Mahape M.I.D.C.,
Navi Mumbai - 400 710, India.
Tel. No.: +91 22 27781889
Fax No.: +91 22 27781884</td><td colspan="2">Bioanalytical Facility (Stool Sample Analysis) and Statistical Facility (Microbiome Analysis):
Decode Age,
No. 7, 1, Haudin Rd,
Halasuru, Yellappa Chetty Layout,
Sivanchetti Gardens,
Bengaluru,
Karnataka 560042

Centenarians Life Sciences Pvt Ltd,
No.7, 1, Haudin Rd, Yellappa Chetty
Layout Sivanchetti Gardens, Halasuru,
Bengaluru, Karnataka 560042</td></tr>
<tr><td>Sponsor</td><td colspan="4">Water and Light Applications India Private Limited,
142, 6A, Kalpataru Estate,
JV Link Road,
Andheri East,
Mumbai 400 093, India.</td></tr>
<tr><td>Sponsor representative</td><td colspan="4">Mr. Madhusudan Rajagopalan (Director)
Water and Light Applications India Private Limited,
142, 6A, Kalpataru Estate,
JV Link Road,
Andheri East,
Mumbai 400 093, India.</td></tr>
</table>

GCP compliance statement	The study has been conducted as per the protocol, GCP guidelines, and applicable SOPs of Raptim Research Pvt. Ltd. The Investigator and the Sponsor have accepted the responsibility for the scientific correctness of the study and validity of the data produced in this CSR (clinical study report).
CSR version and date	Version: 00, 05/02/25

1.2 COMPLIANCE STATEMENT AND SIGNATURES – CRO

We confirm that the present study has been conducted as per the approved protocol in compliance with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, 64th World Medical Association – General Assembly, Fortaleza, Brazil, October 2013)[1], Integrated Addendum to ICH E6(R1): Guideline For Good Clinical Practice E6 (R2), Current Step 4 version dated 9 November 2016[2], New Drugs and Clinical Trials (Amendment) Rules, 2022 [Gazette notification G.S.R.227 (E) dated 19.03.2019, G.S.R.605 (E), dated 31.08.2021 G.S.R 778 (E) dated 14 Oct 2022 and G.S.R. 364 (E), dated 11.05.23], Ministry of Health and Family Welfare, Government of India[3], Guidelines for Bioavailability and Bioequivalence Studies, Central Drug Standard Control Organization, Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India, New Delhi, (March 2005)[4], National Ethical Guidelines for Biomedical and Health Research Involving Human Participants, Indian Council of Medical Research, 2017[5] and other applicable regulatory requirements.

We hereby agree that the present clinical study report (CSR) has been prepared as per the ICH-E3 guidelines[9]. We have read this CSR and confirm to the best of our knowledge that it accurately describes the conduct and results of the present study.

Clinical Investigator Dr. Yashvant Khaire, M.B.B.S., Diploma in Anesthesia Raptim Research Pvt. Ltd., Navi Mumbai, India	05/02/25 **Signature and date**
Bioanalytical Investigator Dr. Milind Bagul, Ph.D. (Head-Analytical Services) Raptim Research Pvt. Ltd., Navi Mumbai, India	05/02/25 **Signature and date**
Chief Controller Data Processing Services Dr. Chandrashankar Gupta, M. Sc., Ph.D. (Statistics) Raptim Research Pvt. Ltd., Navi Mumbai, India	For 05/02/25 **Signature and date**
Head of Quality Assurance Ms. Usha Ramakrishnan, B. Pharma Raptim Research Pvt. Ltd., Navi Mumbai, India	05/02/25 **Signature and date**

2.0 SYNOPSIS

Name of the Sponsor: Water and Light Applications India Private Limited	Individual study table referring to the part of the dossier Volume: Page :	*(For national authority use only)*
Name of the finished product: Analemma Water		
Name of the active ingredient: Analemma Water		

Study Title:

A double blinded, balanced, randomized, two treatment, two period, preliminary microbiome study of Analemma Water and assess the NAD+ assay from whole blood pre and post treatment of Analemma Water in normal, healthy, adult, human subjects

Study Design:

A double blinded*, balanced, randomized, two treatment, two period, preliminary microbiome study and assess the NAD+ assay.

*Person involved in product handling, dispensing and dosing were not involved in particular assessment which causes biasness. e.g., AE monitoring and management. Subject participating in the study were also blinded about the treatment (Test/ Placebo) to be administered.

Investigators:

Clinical Investigator: Dr. Yashvant Khaire, M.B.B.S., Diploma in Anesthesia	**Raptim Research Pvt. Ltd.,** PAP-213, PAP-A-218 and PAP-A-219 (Screening Facility) A-226 (Clinical Unit); A-242 (Biostatistical Unit); T.T.C., Industrial Area, Mahape M.I.D.C., Navi Mumbai - 400 710, India. Tel. No.: +91 22 27781889 Fax No.: +91 22 27781884
Clinical Co-Investigator: Dr. Yagnesh Tadvi, M.B.B.S. Dr. Raviraj Jagdhani, M.B.B.S, M.D (Pharmacology)	
Bioanalytical Investigator Dr. Milind Bagul, Ph.D.	
Chief Controller Data Processing Services Dr. Chandrashankar Gupta, M. Sc., Ph.D. (Statistics)	
Head of Quality Assurance: Ms. Usha Ramakrishnan, B. Pharm.	

Study Center(s): **Screening and Clinical Facility and Statistical Facility (NAD study):** Raptim Research Pvt. Ltd., PAP-A-218 and PAP-A-219 (Screening Facility) A-226 (Clinical Unit); A-242 (Biostatistical Unit); T.T.C., Industrial Area, Mahape M.I.D.C., Navi Mumbai - 400 710, India. Tel. No.: +91 22 27781889 Fax No.: +91 22 27781884	**Bioanalytical Facility (Stool Sample Analysis) and Statistical Facility (Microbe Analysis):** Decode Age, No. 7, 1, Haudin Rd, Halasuru, Yellappa Chetty Layout, Sivanchetti Gardens, Bengaluru, Karnataka 560042 Centenarians Life Sciences Pvt Ltd, No.7, 1, Haudin Rd, Yellappa Chetty Layout Sivanchetti Gardens, Halasuru, Bengaluru, Karnataka 560042

Name of the Sponsor: Water and Light Applications India Private Limited	Individual study table referring to the part of the dossier Volume: Page :	*(For national authority use only)*
Name of the finished product: Analemma Water		
Name of the active ingredient: Analemma Water		

Publications: None

Study Period:

Phase		***Initiation***	***Completion***
Screening		05/06/24	08/06/24
Clinical		08/06/24	13/09/24
Bioanalysis	Day 00	09/06/24	
	Day 96	13/09/24	
Statistical analysis		09/10/24	09/10/24

Study Objectives:

Primary Objective:

To ascertain the preliminary microbiome study of Analemma Water on human health.

To assess the NAD+ assay from whole blood pre and post treatment of Analemma Water in comparison with Placebo drinking water.

Secondary Objective:

- To assess safety of the Analemma Water in comparison with the placebo drinking water.

Methodology:

Based on pre-study examinations performed within 21 days prior to check-in and verification of compliance with the inclusion and exclusion criteria, eligible subjects were checked into the clinical pharmacology unit (CPU) and were provided Analemma Water or Placebo drinking water.

Baseline Day -1 Activities

The activities including biometric identification, obtaining study specific written informed consent (Only during baseline day 0 activities), medical and medication history, urine alcohol test, urine screen for drugs of abuse, physical examination, vital signs measurements (blood pressure, pulse rate, body temperature and respiratory rate), assessing general well-being since last visit and evaluation of inclusion and exclusion criteria were performed one-day prior (Day 0) to consumption of Analemma Water or Placebo drinking water.

Blood and Stool sample were collected from each subject for preliminary microbiome study and NAD+ assay.

Subjects were report one day prior to consumption of Analemma Water or Placebo drinking water.

Following were the study subject visit details:

Period	Day	Visit No.	Activity
Period I	Day 0	01	▪ Blood sample were collected ▪ Stool sample were collected ▪ Sufficient volume of Analemma Water or Placebo drinking water was provided to the subjects to consume daily from day 1 to day 30.

Name of the Sponsor: Water and Light Applications India Private Limited	Individual study table referring to the part of the dossier Volume: Page :	*(For national authority use only)*
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	Day 10	02	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 20	03	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 31	04	▪ Water consumption compliance check ▪ Safety Monitoring ▪ Blood sample were collected ▪ Stool sample were collected. ▪ Remaining quantity of Analemma Water or Placebo drinking water was taken back from the subject.
Wash-out Period	Day 31 to Day 35		
Period II	Day 36	05	▪ Blood sample were collected ▪ Stool sample were collected ▪ Safety Monitoring ▪ Sufficient volume of Analemma Water or Placebo drinking water was provided to the subjects to consume daily from day 36 to day 96.
	Day 46	06	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 56	07	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 66	08	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 76	09	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 86	10	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 96	11	▪ Water consumption compliance check ▪ Safety Monitoring ▪ Blood sample were collected ▪ Stool sample were collected. ▪ Remaining quantity of Analemma Water or Placebo drinking water was taken back from the subject. ▪ Post-study Safety Assessments

Period I: From day 1 to day 30, sufficient volume of placebo drinking water was provided to the subjects. Subject was instructed to consume minimum 1.5-liter water daily as per the randomization schedule.

Subjects were instructed to record the details (Quantity, date and time) of water consumption in the subject diary from day 01 to day 30.

Period II: From day 36 to day 96, sufficient volume of Analemma water was provided to the subjects as per the randomization schedule. Subject was instructed to consume minimum 1.5 liter water daily.

Subjects were instructed to record the details (Quantity, date and time) of water consumption in the subject diary from day 36 to day 96.

Name of the Sponsor: Water and Light Applications India Private Limited	Individual study table referring to the part of the dossier Volume: Page :	*(For national authority use only)*
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For appropriate use of Analemma Water below mentioned Procedure was followed for appropriate use of Analemma Water.

Before you start using this product:
After receipt first checked this product for any fractures. Assured that the Water Tube was totally undamaged before every use and Never use the Water Tube.

Drinking the coherent water:
Subject instructed to use the Water Tube exclusively to produce coherent drinking water for human consumption: water of good quality that has already been filtered and purified and that is free from chemical and/or biological pollution.

All subjects were visited to facility for water consumption compliance check on Day 10, Day 20 and Day 31 during period I and on Day 36, Day 46, Day 56, Day 66, Day 76, Day 86 and Day 96 during period II. Subjects were allowed to visit the facility ± 2 days from above scheduled days.

Blood Sample Collection
A total of 4 blood samples (5.0 mL each) per participant were collected in pre-labeled vacutainers containing K_3EDTA as an anticoagulant during the study.

1. First Baseline Blood Sample was collected at Baseline day (Day 0) during period I.
2. Second Blood Sample was collected on Day 31 during period I.
3. First Baseline Blood Sample was collected at Baseline day (Day 36) during period II.

Second Blood Sample was collected on Day 96 during period II.

Total blood loss for a subject during the study did not exceed 40.0 mL for male subjects and 44.0 mL for female subjects.

Stool Sample Collection
The sample was collected and stored in a Stool DNA stabilizer solution tube by lnvitek
A total of 4 stool samples per participant were collected during the study.

1. First Baseline Sample was collected at Baseline day (Day 0) during period I.
2. Second Sample was collected on Day 31 during period I.
3. First Baseline Sample was collected at Baseline day (Day 36) during period II.
4. Second Sample was collected on Day 96 during period II.

For Women
Collected before or after your menstrual period to prevent potential contamination.

Name of the Sponsor: Water and Light Applications India Private Limited	Individual study table referring to the part of the dossier Volume: Page :	*(For national authority use only)*
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After Returning to Normal Routine
If any of the above conditions apply, wait until your gut microbiome stabilizes post-recovery or post-exposure. Then you can proceed with the test.

Preventing Sample Contamination
Before collecting the stool sample, subjects empty their bladder. Urine or blood In the stool sample can lead to inaccurate test results.

Stool sample was shipped at 2°C to 8°C to Centenarians Life Sciences Pvt Ltd. for Microbe Analysis.
• After registration, we'll schedule a pickup.
• Place the tube in the test box, put it in the return cover and seal it.

Metagenomic sample processing at Decode Age laboratory:

The human microbiome was tested here using Shotgun metagenomic sequencing. The samples and DNA were stored in -20°C to ensure no more microorganism growth in the samples.

Methodology
Processing of Collected Stool Sample
Detailed protocol for processing collected stool samples, including any extraction, preservation, or preparation steps

Sample receiving: When the samples were received in the lab, its weight, colour was noted along with date and time of receiving. If there is any sign of contamination or leak and less quantity, it was rejected.

DNA extraction: DNA extracted using Qiagen Fast Stool Mini kit was used to extract DNA according to the manufacturer's protocol.

Quality analysis: Nanodrop, qubit and gel electrophoresis was conducted on the eluted DNA using standard procedure.

Library preparation: Sample library was prepared using Native Barcoding Kit 96 V14 by attaching unique barcodes and pooling into batches.

Nanopore sequencing: The DNA library was loaded onto nanopore flowcell (v: R10.4.1).

The stool sample processing here in the lab was divided into two major steps. First step was DNA extraction where the stool sample was processed aseptically through Qiagen Fast stool mini kit. The steps were followed as manufacturer‟s instructions given in the kit protocol. The quality of the DNA was checked with nanodrop reading using standard Nanodrop, qubit and ratios of 260/280 (1.7 to 2.0) and 260/230 (2.0-2.2) were observed to ensure good quality DNA goes for sequencing.

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Once we have a good quality DNA per sample, it was then processed for the library preparation step for long read sequencing. The standard steps involved in this process were DNA end preparation, barcode ligation and library pooling. The samples were labelled with a unique barcode to ensure that it was identified during analysis when mixed more samples together during sample pooling. The prepared and pooled library then was loaded onto the flow cell for sequencing. The most common run time for a sequencing machine was around 12 hours depending upon the number of samples and data needed per sample.

Stool samples were stored in a -20 deep freezer after processing.
The data was transferred to the bioinformatics department for further analysis.

Blood Sample Processing

Collected whole blood sample were divided into three aliquots as mentioned below into pre-labeled polypropylene tubes, as early as possible and were stored in the deep freezer maintained at -2°C to 8°C immediately after sample collection:
Aliquot 1: 2.0 mL of whole blood sample was transferred into aliquot 1.

Aliquot 2 (Analytical): 1.5 mL of whole blood sample was transferred into aliquot 2.
Aliquot 3 (Replicate): 1.5 mL of whole blood sample was transferred into aliquot 3.
Transfer of Aliquots:
Aliquot 1 was shipped at 2°C to 8°C to the Pathology Lab of Raptim Research Pvt. Ltd. (For RBC count)
Aliquot 2 & 3 was shipped at 2°C to 8°C to the bioanalytical facility of Raptim Research Pvt. Ltd. (A-242). (NAD+ assay)
Note:

- Aliquot 2 & 3 were transferred on the same day as collection for fresh analysis to the bioanalytical facility. In case fresh analysis was not possible on the same day, it was not postponed beyond 1 day.

RBC count provided prior to sample transfer to the bioanalytical facility.

Number of subjects planned and analyzed:

A total of 10 normal, healthy, adult human subjects were planned and enrolled in the study. Out of these, 09 subjects completed the study.
Subject No.03 withdraw from the study due to personal reason from period I.
Samples of 08 subjects were considered for Test Product and Samples of 09 subjects were considered for Placebo of NAD+ study.
Samples of 09 subjects were considered for Microbiome Analysis

Main Inclusion Criteria:

Normal, healthy, adult, human subjects, 25 to 40 years (both inclusive) of age with a body mass index (BMI) in the range of 18.50 to 29.99 kg/m^2 (both inclusive).

Name of the Sponsor: Water and Light Applications India Private Limited	Individual study table referring to the part of the dossier Volume: Page :	*(For national authority use only)*
Name of the finished product: Analemma Water		
Name of the active ingredient: Analemma Water		

Identity of Investigational Products (IPs):

Parameter	Test Product (A)	Placebo (B)
Product name	Analemma Water (As per protocol)	Placebo drinking water
Manufactured by	Water and Light Applications India Private Limited (As per protocol)	Water and Light Applications India Private Limited (As per protocol)

Study Duration:

The duration of the subject participation was 98 days including washout period of 05 days between consecutive dosing.

Statistical Analysis (NAD+ study):

Applied the Change from baseline approach on the respective parameters data and presented accordingly.

Appropriate statistical test were performed on all dependent variables data (i.e., test parameters data) to test significance among the before and after consumption of the coherent water.
P values greater than 0.05 were considered statistically non-significant.

Safety Analysis Criteria:

Safety and tolerability was assessed in terms of adverse events (AEs), serious adverse event (SAE) if any, or any illness requiring administration of other medication(s) during the study, vital signs and laboratory assessments were performed during the entire course of the study. Adverse events were evaluated based on frequency, severity grades, causality and outcome.

Results of Total NAD assessment based on whole blood concentration data of Analemma water are summarized below in Table 2.1

Table 2.1: Mean Summary table of Analemma Water

Mean ±SD (CV %)			
Placebo (C)		Test Product (D)	
Day 0 (N=10)	Day 31 (N=09)	Day 36 (N=09)	Day 96 (N=08)
3044.49 ± 463.27 (15.22)	2559.30 ± 1843.43 (72.03)	8372.76 ± 1026.64 (12.26)	10033.75 ± 1956.77 (19.50)

N- Number of evaluated subjects;

For checking normality we used Shapiro-Wilk Test:

1. **Period 1 (Day 0 vs. Day 31)**:

   - Day 0 concentration data (baseline) follows a normal distribution ($p = 0.8777$).
   - Day 31 concentration data does not follow a normal distribution ($p < 0.0001$).
   - As a result, the baseline-corrected concentration data for the placebo (Day 31 - Day 0) also does not follow a normal distribution ($p < 0.0001$).

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2. **Period 2 (Day 36 vs. Day 96)**:

   - Both Day 36 (baseline) and Day 96 concentration data follow a normal distribution (p = 0.1116 and p = 0.4408, respectively).
   - Therefore, the baseline-corrected concentration data for the test water (Day 96 - Day 36) follows a normal distribution (p = 0.5120).

For Period 1 (Day 0 vs. Day 31) data does not follow normal distribution. So, we used Wilcoxon signed-rank test and For Period 2 (Day 36 vs. Day 96) data follow normal distribution. So, we used Paired T-test and the same results mentioned below:

1. **Placebo (Day 31 - Day 0)**:

   - **Wilcoxon signed-rank test**: p = 0.1289 (non-significant).
   - This indicates no significant difference between baseline and post-treatment concentration levels for the placebo.

2. **Test Water (Day 96 - Day 36)**:

   - **Paired T-test**: p = 0.0200 (significant).
   - This suggests a significant improvement or change in NAD+ concentration after consuming Analemma Water compared to the baseline.

For Comparison of Placebo (Day 31 - Day 0) and Test Water (Day 96 - Day 36), we used Wilcoxon Rank-Sum Test.

Comparison Between Treatments:

- **Wilcoxon Rank-Sum Test** (non-parametric test for two independent samples):
  - p = 0.0071 (significant).
  - This indicates a statistically significant difference between the baseline-corrected concentrations of the test water (Analemma Water) and placebo. The test water showed a greater effect than the placebo.

Safety Results:

There were no SAEs reported during the study. Overall, 02 AEs were reported during the study. Out of which, no AE was reported during the study periods and all the 02 AEs were reported during post-study safety assessments. Out of 02 AEs 01 was mild and 01 was moderate in intensity. Both the AEs were resolved completely.

Overall Conclusion:

- Analemma Water showed a significant improvement in NAD+ concentration from baseline to post-treatment (p = 0.0200), whereas the placebo showed no significant change (p = 0.1289). This suggests that Analemma Water may have a positive effect on NAD+ levels.

- The significant difference between Analemma Water and placebo (p = 0.0071) further strengthens the evidence that Analemma Water has a favorable impact compared to

Name of the Sponsor: Water and Light Applications India Private Limited	Individual study table referring to the part of the dossier Volume: Page :	*(For national authority use only)*
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placebo drinking water.

This study provides preliminary evidence that Analemma Water may positively affect NAD+ levels.

There were no SAEs observed during the study and no unresolved AEs with either the test product or the placebo. The water treated with test product was well tolerated during the study and was found safe for consumption**.**

Version: 00, 05/02/25

3.0 TABLE OF CONTENTS

LIST OF TABLES

4.0 LIST OF ABBREVIATIONS

Abbreviation	Full Form
AE	Adverse Event
ANOVA	Analysis of Variance
BA	Bioanalysis
BMI	Body Mass Index
CI	Confidence Interval
COA	Certificate of Analysis
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
CV	Curriculum Vitae
DCGI	Drugs Controller General of India
ECG	Electrocardiogram
GCP	Good Clinical Practice
GMR	Geometric Mean Ratio
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICH	International Council on Harmonisation
ICMR	Indian Council of Medical Research
IEC	Independent Ethics Committee
IP	Investigational Product
IU	International Unit
Ln	Natural logarithm
MD	Doctor of Medicine
OTC	Over The Counter
QC	Quality Control
R/M	Routine and Microscopy
SAE	Serious Adverse Event
SAS	Statistical Analysis System
SD	Standard Deviation
SIS-ICF	Subject Information Sheet and Informed Consent Forms

5.0 ETHICS

5.1 INDEPENDENT ETHICS COMMITTEE (IEC)

The study protocol version 00, dated 15/04/24, Subject Information Sheet and Informed Consent Form (SIS-ICF) version 00 (dated 24/04/24; English, Hindi, and Marathi), Investigational Product (IP) Information and other protocol related documents were reviewed and approved by Ethicare Ethics Committee in the meeting held on 28/04/24.

The IEC approved protocol is provided in **Appendix 16.1.1**. The IEC composition, CVs of IEC members, sample copies of study specific ICFs (English, Hindi, and Marathi), and IEC approval letter are provided in **Appendix 16.1.3**.

5.2 ETHICAL CONDUCT OF THE STUDY

The study was conducted in compliance and accordance with the ethical principles that have their origins in the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, 64th World Medical Association – General Assembly, Fortaleza, Brazil, October 2013)[1], Integrated Addendum to ICH E6(R1): Guideline For Good Clinical Practice E6 (R2), Current Step 4 version dated 9 November 2016[2], New Drugs and Clinical Trials (Amendment) Rules, 2022 [Gazette notification G.S.R.227 (E) dated 19.03.2019, G.S.R.605 (E), dated 31.08.2021 G.S.R 778 (E) dated 14 Oct 2022 and G.S.R. 364 (E), dated 11.05.23], Ministry of Health and Family Welfare, Government of India[3], Guidelines for Bioavailability and Bioequivalence Studies, Central Drug Standard Control Organization, Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India, New Delhi, (March 2005)[4], National Ethical Guidelines for Biomedical and Health Research Involving Human Participants, Indian Council of Medical Research, 2017[5] and other applicable regulatory requirements.

In order to achieve and comply with the ethical principles mentioned in above guidelines: clinical monitoring was performed; integrity of data was maintained during its generation and only quality assurance approved data were used for estimation of NAD study, Microbe Analysis and assessment of safety in the present study.

5.3 SUBJECT INFORMATION AND CONSENT

All the subjects screened for the study received information both verbally and in written form in subject‟s respective vernacular (Marathi or Hindi) language regarding the purpose and procedures involved in the screening. Screening procedures were performed only after obtaining screening consent from the subjects.

At the time of enrollment in the study, the subjects were selected from those qualified during the screening process and received information both verbally and in written form in Hindi or Marathi language explaining the purpose and nature of the study and its procedures as well as potential risks and benefits (if any) associated with IPs as per the SIS-ICF. The subjects were provided enough time

and opportunity to read the SIS-ICF. The subjects were encouraged to ask questions and clarify their doubts before signing the ICF in the presence of an investigator or qualified medical personnel of Raptim Research Pvt. Ltd., India prior to participation in the study. Subject"s signature was obtained in the respective vernacular ICF. The sample copy of IEC approved study specific SIS-ICF is provided in **Appendix 16.1.3**.

A copy of the signed and dated study specific SIS-ICF was provided to the individual subject.

6.0 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The below table describes administrative structure of the study:

Table 1: Study investigators and administrative structure

<table>
<tr><th colspan="2">Contract Research Organization</th></tr>
<tr><th>Name</th><th>Address</th></tr>
<tr><td>Clinical Investigator:
Dr. Yashvant Khaire, M.B.B.S., Diploma in Anesthesia</td><td rowspan="4">Raptim Research Pvt. Ltd.,
Clinical Pharmacology Unit (Clinic and Pathology): A-226;
Screening Facility (PAP-213, PAP-A-218 and PAP-A-219);
Biostatistical Unit: A-242;
T.T.C., Industrial Area, Mahape M.I.D.C.,
Navi Mumbai – 400 710, India.
Tel. No.: +912227781889;
Fax No.: +912227781884</td></tr>
<tr><td>Clinical Co-Investigator:
Dr. Yagnesh Tadvi, M.B.B.S.
Dr. Raviraj Jagdhani, M.B.B.S, M.D (Pharmacology)</td></tr>
<tr><td>Bioanalytical Investigator
Dr. Milind Bagul, Ph.D.</td></tr>
<tr><td>Chief Controller Data Processing Services
Dr. Chandrashankar Gupta, M. Sc., Ph.D. (Statistics)</td></tr>
<tr><td>Head of Quality Assurance:
Mrs. Usha Ramakrishnan, B. Pharm.</td><td></td></tr>
<tr><th colspan="2">Independent Ethics Committee</th></tr>
<tr><td colspan="2">Ethicare Ethics Committee,
Shop No. 9, Ground Floor, Patidar Complex,
Kannamwar Nagar - 2,Vikhroli (E),
Mumbai – 400083, India.
Tel. No.: +91 22 2577 8957
Email: contactethicare@yahoo.com</td></tr>
<tr><th colspan="2">Sponsor’s Representative</th></tr>
<tr><td colspan="2">Mr. Madhusudan Rajagopalan (Director)
Water and Light Applications India Private Limited,
142, 6A, Kalpataru Estate,
JV Link Road,
Andheri East,
Mumbai 400 093, India.</td></tr>
<tr><th colspan="2">Clinical Pathology Laboratory</th></tr>
<tr><td colspan="2">In-house Clinical Pathology Laboratory,
Raptim Research Pvt. Ltd.,
A-226, T.T.C. Industrial Area, Mahape M.I.D.C.,
Navi Mumbai – 400710, India.
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Fax No.: +912227781884</td></tr>
</table>

The list of investigators and other important participants in the study and their CVs is provided in **Appendix 16.1.4.** The signature of investigators is provided in **Appendix 16.1.5**.

7.0 INTRODUCTION

Analemma Water

Water, H_2O, is a broadband absorber of electromagnetic radiation (EM). The water in our atmosphere absorbs almost 70% of the sun's/cosmic EM like UV light, infrared and microwaves. This makes it possible for (human) life to survive on our planet's surface. Furthermore, water is also involved in various important intra and extra cellular processes and in maintaining our DNA structure. Without water, we humans would not be living on earth

Water has tremendous capacity. It works as a recipient and transmitter of information in the form of light (EM). Research shows that if water molecules are arranged in a liquid crystalline structure, referred to as coherent water, it resonates with electromagnetic fields in unison. Like the antenna of a radio that needs to be set on the right frequency in order to receive and transmit a clear signal. Because of the possible variety in crystalline structures, water can resonate with a myriad of electromagnetic waves. Since water is involved in almost all biological processes in the human body, is seems likely that this „antenna" function of coherent water, plays a significant role in supporting or even initiating these processes.

Unfortunately, the water we humans consume and contain in our bodies is less and less in this coherent state. The crystalline structure is disturbed by the progressive exposure to rapidly increasing amounts of EM radiation (like Wi-Fi, cell phone and satellite communication) but also to chemical pollution due to the enormous amount of air pollution, nuclear radiation, and chemicals (like pesticides). This causes the H2O molecules to move in a chaotic and irregular manner, losing its coherent quality.

Analemma water is normal water of which the H_2O molecules have been reconstructed into the original coherent state. Since our body consists of 99.1 % H_2O molecules, taking up 70% of our body weight, and water being involved in almost every biological and physiological process, the hypothesis is that changing the water structure and its stability has an influence on the quality of this process.

Analemma is the brand name under which the full spectrum coherent water developed the parent company Water and Light B.V is marketed globally. Earlier, in India, the brand name Somarka was used but that has been harmonized as Analemma since January 2024.

Microbiome and Health

The microbiota inhabiting the gastrointestinal tract play essential roles in regulating host physiology. External factors such as lifestyle choices and dietary habits are widely acknowledged as significant influencers of the composition of both the gut and oral microbiomes. Despite being one of the most consumed food items, the potential influence of drinking water on the microbiome remains relatively understudied. Traditional water treatment facilities typically carry out processes such as filtration, sedimentation, disinfection, and flocculation, enabling the evaluation of microbial levels present in the water. (Chao et al., 2013; Dodd, 2012; Loubet et al., 2016). However, certain microorganisms have the potential to endure and multiply within drinking water, with bacterial quantities approximated to range from 10^6 to 10^8 cells per liter (Hammes et al., 2008; Hong et al., 2010). A

recent study on 3413 and 3794 individuals from US and UK populations, respectively has shown distinct microbial signatures associated with source and intake of drinking water (Vanhaecke et al., 2022). A study carried out by Lugil et al in 2022 by exhaustive shotgun metagenomics analysis of the tap water microbiome highlighted the occurrence of a high genetic biodiversity of the microbial communities residing in fresh water and the existence of a conserved core tap water microbiota largely represented by novel microbial species, representing microbial dark matter (Lugli et al., 2022). This study also investigated the effect of long-term consumption and showed that it can result in horizontal transmission and colonization of water bacteria in the human gut.

Similarly, recent studies have shown the effect of pH of drinking water and its association with Type 1 Diabetes and glucose regulation mediated by microbiota (Hansen et al., 2018; Sofi et al., 2014; Wolf et al., 2014). N-nitrosamines (NAs) are an emerging group of disinfection by-products that occur as a mixture in drinking water. Although the potency of the individual NA components in drinking water is negligible, their combined effect was rarely reported. Zhu et al., 2019 found that the body weight gains, and the triglyceride (TG) levels increased significantly in male rats. Firmicutes/Bacteroidetes and the obesity-related taxa including *Alistipes*, *Ruminococcus* were found to be enriched. (Zhu et al., 2019). Similar studies focusing on dichloroacetonitrile (DCAN) and Chlorinated drinking water have shown perturbation of gut microbiome (Martino, 2019; Xue et al., 2020).

Overall, recent research underscores the importance of considering drinking water as a significant factor in shaping gut microbial composition and, consequently, human health. By gaining insights into the dynamic interactions between water and the gut microbiome, scientists are better equipped to develop strategies for promoting a healthy microbial balance and mitigating the risk of associated health problems. This growing body of evidence highlights the need for further research and public health initiatives aimed at ensuring access to clean, safe drinking water for maintaining optimal gut health and overall well-being.

Significance of NAD+ and NADH

NAD+ and NADH are essential molecules in our bodies, crucial for various cellular functions. NAD+ serves as a coenzyme involved in numerous enzymatic reactions, playing a pivotal role in cellular metabolism. When NAD+ accepts electrons, it becomes NADH, which carries these electrons to the electron transport chain for ATP production.

Their significance in human health stems from their involvement in critical cellular processes. NAD+ participates in glycolysis, the citric acid cycle, and oxidative phosphorylation, which collectively generate ATP, the cell's energy currency. Additionally, NAD+ is essential for DNA repair, cell signaling, and maintaining genomic stability.

Maintaining a balanced NAD+/NADH ratio is crucial for cellular homeostasis and overall health. Disruptions in this ratio can lead to metabolic dysfunction, impaired cellular energetics, and increased oxidative stress. Studies have linked dysregulation of NAD+ metabolism to various age-related diseases, including neurodegenerative disorders, cardiovascular diseases, and metabolic syndrome.

To enhance NAD+ levels and promote optimal health, several strategies can be employed. Caloric restriction, intermittent fasting, and regular exercise have been shown to upregulate NAD+ synthesis pathways. Additionally, supplementation with NAD+ precursors, such as nicotinamide riboside (NR) or nicotinamide mononucleotide (NMN), can boost NAD+ levels and support cellular function.

The decline of NAD+ and NADH in the human body can have profound effects on health and aging. Reduced NAD+ levels have been associated with diminished mitochondrial function, impaired DNA repair mechanisms, and increased susceptibility to cellular damage. Furthermore, decreased NAD+ availability contributes to mitochondrial dysfunction and cellular senescence, accelerating the aging process and predisposing individuals to age-related diseases.

NAD+ and NADH play indispensable roles in maintaining cellular function and promoting overall health. Strategies aimed at preserving NAD+ levels, such as lifestyle modifications and supplementation, hold promise for mitigating age-related decline and enhancing longevity.

Numerous hereditary and acquired disorders in humans are caused by the depletion of nicotinamide adenine dinucleotide (NAD+), a crucial redox cofactor and the substrate of important metabolic enzymes. Impaired biosynthesis causes primary deficiencies of NAD+ homeostasis, whereas other factors that impact NAD+ homeostasis, like elevated NAD+ consumption or dietary deficits of its vitamin B3 precursors, can cause secondary deficiencies. NAD+ depletion can manifest in a wide variety of pathological phenotypes, ranging from rare inherited defects, characterized by congenital malformations, retinal degeneration, and/or encephalopathy, to more common multifactorial, often age-related diseases.

Based on literature it has been well documented that increasing the NAD+ results in increased capacity to exercise, decreased blood pressure, decreased anti-inflammatory circulating cytokines, increased insulin-stimulated glucose disposal, or decreased fat-free mass. NAD+ levels decrease with age, obesity, and hypertension, which are all risk factors for cardiovascular disease. Increasing NAD+ levels can reduce chronic inflammation, improve mitochondrial function, and enhance oxidative metabolism in vascular cells. NAD+ levels can improve cognitive function and protect against age-related cognitive disorders like Parkinson's disease. Also NAD+ levels can help maintain the integrity of the skin by activating autophagy pathways and clearing damaged cellular proteins.

8.0 STUDY OBJECTIVES

Primary Objective:

To ascertain the preliminary microbiome study of Analemma Water on human health.

To assess the NAD+ assay from whole blood pre and post treatment of Analemma Water in comparison with Placebo drinking water.

Secondary Objective:

- To assess safety of the Analemma Water in comparison with the placebo drinking water.

9.0 INVESTIGATIONAL PLAN

9.1 OVERALL STUDY DESIGN AND PLAN DESCRIPTION

This was a double blinded, balanced, randomized, two treatment, two period, preliminary microbiome study conducted in 10 normal, healthy, adult, human male subjects. Subjects were randomized into two treatment sequence groups.

Study was conducted in following phases based on the activities performed.

Screening phase:
The following screening activities were preformed within 21 days prior to check-in:

Registration/identification in the biometric system, obtaining written informed consent for screening, demographic parameters (gender, race, ethnicity, age, height, weight, body mass index), medical and medication history, physical examination, vital signs measurements (blood pressure, pulse rate, body temperature and respiratory rate), 12-lead ECG recording, laboratory investigations (hematology, biochemistry, serology and urine analysis) and evaluation of inclusion and exclusion criteria. Subjects were given consent and awareness instructions for corona virus disease COVID-19.

Treatment phase (day 0 to day 96):
The following activities were performed from the day of check-in until post-study safety assessments.

Baseline Day 0 Activities and Study Duration
One day prior to consumption of Analemma Water or Placebo drinking water (On Day 0 and Day 36), the activities performed were included biometric identification, obtaining study specific written informed consent (Only during baseline day 0 activities), medical and medication history, urine alcohol test, urine screen for drugs of abuse, physical examination, vital signs measurements (blood pressure, pulse rate, body temperature and respiratory rate), assessing general well-being since last visit and evaluation of inclusion and exclusion criteria.

Blood and Stool sample were collected from each subject for preliminary microbiome study and NAD+ assay.

Study Visits:
Subjects were report one day prior to consumption of Analemma Water or Placebo drinking water.

Following were the study subject visit details:

Period	Day	Visit No.	Activity
Period I	Day 0	01	▪ Blood sample were collected ▪ Stool sample were collected ▪ Sufficient volume of Analemma Water or Placebo drinking water was provided to the subjects to consume daily from day 1 to day 30.
	Day 10	02	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 20	03	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 31	04	▪ Water consumption compliance check ▪ Safety Monitoring ▪ Blood sample were collected ▪ Stool sample were collected. ▪ Remaining quantity of Analemma Water or Placebo drinking water was taken back from the subject.
Wash-out Period	Day 31 to Day 35		
Period II	Day 36	05	▪ Blood sample were collected ▪ Stool sample were collected ▪ Safety Monitoring ▪ Sufficient volume of Analemma Water or Placebo drinking water was provided to the subjects to consume daily from day 36 to day 96.
	Day 46	06	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 56	07	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 66	08	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 76	09	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 86	10	▪ Water consumption compliance check ▪ Safety Monitoring
	Day 96	11	▪ Water consumption compliance check ▪ Safety Monitoring ▪ Blood sample were collected ▪ Stool sample were collected. ▪ Remaining quantity of Analemma Water or Placebo drinking water was taken back from the subject. ▪ Post-study Safety Assessments

Water Restrictions

Consumption of normal drinking water was not allowed during the duration of the study.

Dosing

Period I: From day 1 to day 30, sufficient volume of placebo drinking water was provided to the subjects as per the randomization schedule. Subjects were instructed to consume minimum 1.5 liter water daily.

Subjects were instructed to record the details (Quantity, date and time) of water consumption in the subject diary from day 01 to day 30.

Period II: From day 36 to day 96, sufficient volume of Analemma water was provided to the subjects as per the randomization schedule. Subjects were instructed to consume minimum 1.5 liter water daily.

Subjects were instructed to record the details (Quantity, date and time) of water consumption in the subject diary from day 36 to day 96

Before you start using this product:
After receipt first check this product for fractures. In case of fractures or any other damage, the tube was not used. Before every use, ensure that the Water Tube was totally undamaged.

Drinking the coherent water:
Use the Water Tube exclusively to produce coherent drinking water for human consumption: water of good quality that had already been filtered and purified and that was free from chemical and/or biological pollution. Tap water was only used when this was tap water qualified for human consumption.

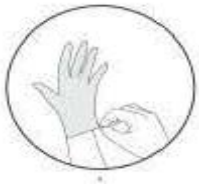
Blood Sample Collection:
A total of 4 blood samples (5.0 mL each) per participant were collected in pre-labeled vacutainers containing K_3EDTA as an anticoagulant during the study.

1. First Baseline Blood Sample was collected at Baseline day (Day 0) during period I.
2. Second Blood Sample was collected on Day 31 during period I.
3. First Baseline Blood Sample was collected at Baseline day (Day 36) during period II.
4. Second Blood Sample was collected on Day 96 during period II.

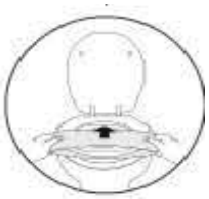
Stool Sample Collection:

The sample was collected and stored in a Stool DNA stabilizer solution tube by lnvitek

Stool Sample Collection Steps:
Step 1
Hygiene First
Wear the provided gloves.

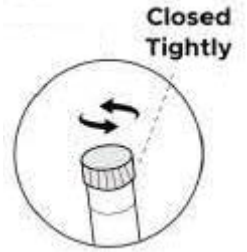


Step 2
Prepare for Collection 1
Lay the biodegradable paper on the toilet seat. Avoiding contact with water



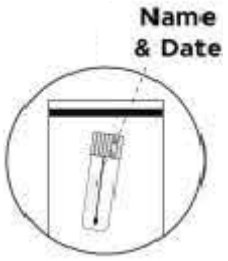
Step 3
Collect the Sample
After a bowel movement. stir the stool with the attached scoop. Scoop two portions into the tube.

Step 4
Secure the Sample:
Seal the tube tightly to prevent leakage or contamination



Step 5
Dispose of the Paper
Tear and flush the paper. If needed, flush again after 15 minutes.

Step 6
Label the Tube and Store
Label with patient's name and date. Put the tube in the clear bag store at room temperature.



Note: To prevent contamination. don't leave the tube open for long, and avoid placing the scoop on surfaces. Ensured that the contents did not spilled, as the liquid inside was crucial for maintaining sample integrity.

A total of 4 stool samples per participant were collected during the study.

1. First Baseline Sample was collected at Baseline day (Day 0) during period I.
2. Second Sample was collected on Day 31 during period I.
3. First Baseline Sample was collected at Baseline day (Day 36) during period II.
4. Second Sample was collected on Day 96 during period II.

For Women
Collected before or after your menstrual period to prevent potential contamination.

After Returning to Normal Routine
If any of the above conditions apply, wait until your gut microbiome stabilizes post-recovery or post-exposure. Then you can proceed with the test.

Preventing Sample Contamination
Before collecting the stool sample, please empty your bladder. Urine or blood In the stool sample lead to inaccurate test results.

Stool sample was shipped at 2°C to 8°C to Centenarians Life Sciences Pvt Ltd. for Microbe Analysis.
• After registration, scheduled a pickup.
• Place the tube in the test box, put it in the return covered and sealed it.

After pickup issues arise, the courier the samples were shipped to below address.
Centenarians Life Sciences Pvt Ltd,
No.7, 1, Haudin Rd, Yellappa Chetty
Layout Sivanchetti Gardens, Halasuru,
Bengaluru, Karnataka 560042

Safety Monitoring:
The safety of subjects was assessed by monitoring for occurrence of any AE as well as vital signs and general well-being during the in-house stay. Vital signs measurements (blood pressure, pulse rate, body temperature, and respiratory rate) and well-being were evaluated at Compliance Check Visit (i.e. on day 10 (Visit 2), day 20 (Visit 3), day 31 (Visit 4), day 36 baseline period II (Visit 5), day 46 (Visit 6), day 56 (Visit 7), day 66 (Visit 8), day 76 (Visit 9), day 86 (Visit 10), day 96 (Visit 11)).

Restriction of fluid and other substances:
Subjects abstained from smoking or chewing tobacco products, alcohol or alcoholic products, xanthine or its derivative containing food or beverages and grapefruit or its juice as per Section 9.3.1 (Inclusion Criteria) and Section 9.3.2 (Exclusion Criteria).

Blood loss:
Total blood loss for a subject during the study did not exceed 40.0 mL for male subjects and 44.0 mL for female subjects.

Post-study safety assessments:
Post-study safety evaluations were performed on day 96. During post-study safety assessments physical examination, vital signs measurements (blood pressure, pulse rate, body temperature and respiratory rate), well-being, and blood sample collection for laboratory analysis [hematology and biochemistry (Serum creatinine, SGOT/AST, SGPT /ALT, serum bilirubin–Total, serum blood urea nitrogen) was performed.

The schedule of study events (Table 2) and a list of laboratory tests performed (Table 3) as part of the screening and post-study safety examinations are presented below.

Table 2: Schedule of Study Events

Procedure	**Screening (within 21 days prior to baseline day 0)**	**Treatment phase**			**Post Study (Day 96) OR Withdrawal / termination / dropout of a subject**
		Baseline Day: **Period I:** Day 0 **Period II:** Day 36	**Water Consumption** **Period I:** Daily from day 1 to day 30 **Period II:** Daily from day 36 to day 96	**Compliance check** **Period I:** Day 10, Day 20 and Day 31 **Period II:** Day 46, Day 56, Day 66, Day 76, Day 86 and Day 96	
Screening consent form	X				
Study specific informed consent		X[#]			
Demographics	X				
Medical and medication History	X	X			
Physical examination	X	X		X	X
Vital signs	X	X		X	X
Well-being		X		X	X
Hematology	X				X
Biochemistry	X				X
Serology	X				
Urine analysis (Routine/Microscopic)	X				
Urine pregnancy test (for female subject)	X				
12 Lead ECG recording	X				
Applicable Inclusion-Exclusion criteria check	X	X			
Urine drug of abuse test		X			
Urine alcohol test		X			
Water Consumption			X	X	
Stool sample		X		X	
Safety monitoring		X		X	X

Note: A washout period of at least 5 days was maintained between each dosing period;
[#]Only during baseline day 0 activities;
Note: Screening was performed on the day of check-in during period I, for few subjects.

Table 3: Safety Laboratory Investigations

<table>
<tr><th rowspan="2">Hematology</th><th rowspan="2">Biochemistry</th><th colspan="2">#Urine analysis</th></tr>
<tr><th>Routine</th><th>Microscopy</th></tr>
<tr><td rowspan="2">• Erythrocyte Count
• Hemoglobin
• WBC Count
• Platelet Count
• Neutrophils
• Eosinophils
• Lymphocytes
• Basophils
• Monocytes</td><td rowspan="2">• Serum Alkaline Phosphatase
• Serum Creatinine
• Serum SGOT/AST
• Serum SGPT/ALT
• Serum Uric Acid
• Serum Blood Urea Nitrogen
• Plasma Glucose (Random)
• Serum Bilirubin - Total</td><td>• Colour
• Appearance
• Reaction pH
• Protein (Albumin)
• Ketone bodies
• Sugar (Glucose)
• Occult Blood
• Urobilinogen</td><td>• Red Blood Cells
• Pus Cells
• Epithelial Cells
• Casts
• Crystals
• Bacteria</td></tr>
<tr><td colspan="2">*Urine Examination for Alcohol Test</td></tr>
<tr><td colspan="2">#Serology</td><td colspan="2">*Urine Examination For Drugs of Abuse</td></tr>
<tr><td colspan="2">• HIV antibodies (I &II)
• HBsAg
• HCV</td><td>• Benzodiazepines (BZO)
• Barbiturates (BAR)
• Tetrahydrocannabinol (THC)</td><td>• Opiate (OPI)
• Cocaine (COC)
• Amphetamine (AMP)</td></tr>
</table>

*At the time of baseline day – 1 activities.
#At screening only

Bioanalysis:
For NAD+ assay, a kit from Sigma Aldrich., "NAD/NADH Quantitation Kit was used

Statistical Analysis:

Applied the Change from baseline approach on the respective parameters data and presented accordingly.

Appropriate statistical test were performed on all dependent variables data (i.e., test parameters data) to test significance among the before and after consumption of the coherent water.

P values greater than 0.05 were considered statistically non-significant.

9.2 DISCUSSION OF STUDY DESIGN

A double blinded, balanced, randomized, two treatment, two period, preliminary microbiome study of Analemma Water and assess the NAD+ assay from whole blood pre and post treatment of Analemma Water was designed in accordance with the reference literature.

9.3 SELECTION OF STUDY POPULATION

The targeted study population was drawn from a local population database of Raptim Research Private Limited, Navi Mumbai, India. Clinical Investigator qualified only those volunteers for entry into the study who met the following inclusion criteria and none of the exclusion criteria at screening and during check-in procedures for each study period.

9.3.1 Inclusion Criteria

A subject fulfilled the following criteria was included in the present study:

- Willing to provide written informed consent for participation in the study, and an ability to comprehend the nature and purpose of the study;
- Willing to be available for the entire study period and to comply protocol requirements;
- Normal, healthy, adult, human subject of 25-40 years (both inclusive) of age;
- Body mass index in the range of 18.50 – 29.99 kg/m^2 (both inclusive);
- Normal health status as determined by baseline medical and medication history, at the time of screening and vital signs measurements and physical examination at the time of screening as well as prior to baseline day visit;
- Normal or clinically non-significant laboratory values as determined by hematological, biochemistry tests and urine analysis;
- Normal or clinically non-significant 12-lead ECG recording;
- Non-smokers;
- Non-alcoholic;
- For female subjects: Negative urine pregnancy test during screening visit.

9.3.2 Exclusion Criteria

A subject with the following criteria was excluded from the study:

- Any medical or surgical conditions, which might significantly interfere with the functioning of gastrointestinal tract and of blood forming organs;
- Significant history or current evidence of malignancy or chronic - infectious, cardiovascular, renal, hepatic, ophthalmic, pulmonary, neurological, metabolic (endocrine), hematological, gastrointestinal, dermatological, immunological or psychiatric diseases, or organ dysfunction;
- Any major illness or hospitalized within 90 days prior to the baseline day visit;
- Have had a fever, viral, or bacterial infection within the past 7 days;
- Have taken antibiotics within the last two weeks;
- Have consumed alcohol or recreational drugs in the last 24 hours;
- Requiring medication for any ailment having enzyme-modifying activity within one month prior to baseline day visit (day-0) and throughout the study;
- Use of any depot injection or an implant of any drug within 3 months prior to baseline day visit (day-0) and throughout the study;
- Use of any prescribed medication (including herbal medicines and vitamin supplements) or OTC products within 30 days prior to baseline day visit (day-1) and throughout the study;
- Vaccinated 7 days prior to baseline day visit (day-0) and willing to get vaccinated during the study;
- History or presence of significant gastric and/or duodenal ulceration;
- Use of any recreational drug or history of drug addiction;
- Participated in any clinical investigation requiring repeated blood sampling or have donated blood in past 90 days prior to baseline day visit;
- Positive urine alcohol and urine drug of abuse tests during baseline day visit;
- Reactive test for Human Immunodeficiency Virus (HIV) type I/II antibodies or Hepatitis B surface antigen (HBsAg) or Hepatitis C virus antibodies;

- Lactating or nursing female subjects;
- Female subjects using hormonal contraceptive (either oral/implants);
- History of difficulty in accessibility of veins in arms.

9.3.3 Removal of Subjects from the Study or Assessment

Subjects were to be removed from the study or study evaluations for any of the following reasons:

- Dropout: A subject fails to return to the clinical facility for any of the remaining periods after participating in the first period.
- Withdrawal: Subject‟s decision to withdraw his/her voluntary participation, anytime during the study period.
- Termination: The clinical investigator may terminate a subject from the study for any of the valid reasons, which is appropriate in view of the safety and well-being of subject, GCP principles or objectives of the study, in particular for but not limited to:
  - ✓ Any serious adverse event (SAE) during the study;
  - ✓ Any illness requiring surgical procedures or administration of other medication(s) during the study, which could impact the PK profile of investigational product;
  - ✓ Protocol violation or noncompliance to the study protocol by the subject;
  - ✓ Further continuation in the study exposes the subject to potential AE that may prove harmful to the subject;
  - ✓ Sponsor‟s decision to terminate the study based on safety issues related to the investigational product;

The reason for subject discontinuation was documented in the subject‟s case report form (CRF).

9.4 TREATMENTS

9.4.1 Treatments Administered

Details of treatments administered are provided in below table

Table 4: Treatments administered

Parameter	Test Product (A)	Placebo (B)
Dosage form	Analemma Water (As per protocol)	Placebo drinking water
Route	Oral	Oral
Dose and Mode of administration	Analemma Water was provided to consume minimum 1.5 liter water Period II: Daily from day 36 to day 96	Placebo drinking water was provided to consume minimum 1.5 liter water Period I: Daily from day 1 to day 30
Dosing date in period I	08/06/24	
Dosing date in period II	14/07/24	

9.4.2 Identity of Investigational Products

Investigational products (IPs) were supplied by the Sponsor and received by the pharmacist at Raptim Research Pvt. Ltd., India. IPs received were verified intact condition of packaging. The identities of the IPs are provided in below table.

Table 5: Identity of investigational products (IPs)

Parameter	Test Product (A)	Placebo (B)
Product name	Analemma Water (As per protocol)	Placebo drinking water
Manufactured by	Water and Light Applications India Private Limited (As per protocol)	Water and Light Applications India Private Limited (As per protocol)

On study completion, the quantity of investigational product received (test product and reference product) from the Sponsor, dispensed, undispensed quantity, and unused dispensed quantity were reconciled and retained as per in-house SOP for the estimation of the balance quantity of IP. All the unused IPs were returned to the pharmacy and stored in the same formulation cabinet with that of the undispensed IPs.

COAs and IP Accountability Record of the test product and the reference product are provided in **Appendix 16.1.6.**

9.4.3 Method of Assigning Subjects to Treatment Groups

As per the study design, (C and D) randomization schedule was generated by a Biostatistician at Raptim Research Pvt. Ltd., India by PROC PLAN procedure (such that the design being balanced over the period and sequence combination) using Statistical Analysis Software SAS® Version 9.4 (SAS Institute Inc., U.S.A.).

Subjects were allocated sequential numbers starting from 01 on the day of check-in for period I and were assigned randomization sequence as per their number stated in randomization schedule.

The randomization schedule generated is provided in **Appendix 16.1.7**.

9.4.4 Selection and Timing of Dose for Each Subject

Period I: From day 1 to day 30, sufficient volume of placebo drinking water was provided to the subjects as per the randomization schedule. Subjects were instructed to consume minimum 1.5 liter water daily.

Subjects were instructed to record the details (Quantity, date and time) of water consumption in the subject diary from day 01 to day 30.

Period II: From day 36 to day 96, sufficient volume of Analemma water was provided to the subjects as per the randomization schedule. Subjects were instructed to consume minimum 1.5 liter water daily.

Subjects were instructed to record the details (Quantity, date and time) of water consumption in the subject diary from day 36 to day 96.

9.4.5 Blinding

The present study was designed as a blinded. The study subjects, the Clinical Investigator, the study staff involved in study activities, bio-statistician, bio-analyst and the sponsor were blinded for the treatment administered to subject.

Only pharmacists and assistant pharmacists who were responsible for dispensing investigational products were having access to the randomization schedule. They

were not involved in any other study-related activities until the completion of the analysis. QA auditors, assigned to monitor IP-related activities such as acceptance, dispensing, and reconciliation, were having access to the randomization schedule but were not involved in other study-related activities like dosing and post-dose activities until the completion of the clinical phase of the study. QA auditors assigned to monitor the bio-analytical phase were not having access to the randomization schedule until the completion of the analysis.
The pharmacist was assign the treatment code (which is an alphabetic code i.e. X or Y or any other notation) for the randomization notation (i.e. if A/B, T/R or any other notation) given in the Randomization schedule generated by the Bio-statistician for test or reference treatment. The treatment code assigned for test and reference was recorded in the "Assignment of treatment code for blinded study design" format which was kept in a sealed envelope in the pharmacy. The photocopy of this format was provided to biostatistician on 05/10/24 after completion of clinical and bioanalytical phase of the study for statistical analysis.

9.4.6 Prior and Concomitant Therapy

Enrolled subjects followed restrictions regarding medications (including herbal medicines and vitamin supplements) or OTC products prior to dosing in period I as mentioned in the exclusion criteria (Section 9.3.2). None of the subjects received concomitant medication during the entire study.

9.4.7 Treatment Compliance

Period I: From day 1 to day 30, sufficient volume of placebo drinking water was provided to the subjects as per the randomization schedule. Subjects were instructed to consume minimum 1.5 liter water daily.

Subjects were instructed to record the details (Quantity, date and time) of water consumption in the subject diary from day 01 to day 30.

Period II: From day 36 to day 96, sufficient volume of Analemma water drinking water was provided to the subjects as per the randomization schedule. Subjects were instructed to consume minimum 1.5 liter water daily.

Subjects were instructed to record the details (Quantity, date and time) of water consumption in the subject diary from day 36 to day 96.

9.5.4 Drug Concentration Measurements

9.5.4.1 Procedures of blood sample and Stool Sample collection and processing

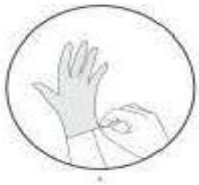
Blood Sample Collection:
A total of 4 blood samples (5.0 mL each) per participant were collected in pre-labeled vacutainers containing K_3EDTA as an anticoagulant during the study.

5. First Baseline Blood Sample was collected at Baseline day (Day 0) during period I.
6. Second Blood Sample was collected on Day 31 during period I.
7. First Baseline Blood Sample was collected at Baseline day (Day 36) during period II.
8. Second Blood Sample was collected on Day 96 during period II.

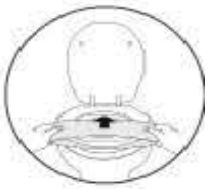
Stool Sample Collection:

The sample was collected and stored in a Stool DNA stabilizer solution tube by lnvitek

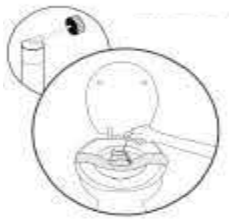
Stool Sample Collection Steps:
Step 1
Hygiene First
Wear the provided gloves.



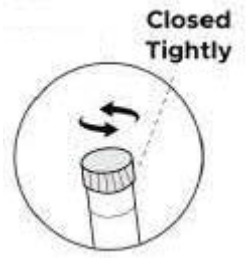
Step 2
Prepare for Collection 1
Lay the biodegradable paper on the toilet seat. Avoiding contact with water



Step 3
Collect the Sample
After a bowel movement. stir the stool with the attached scoop. Scoop two portions into the tube.

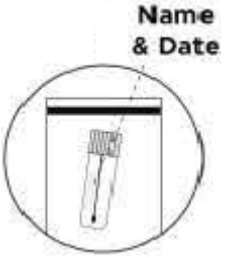


Step 4
Secure the Sample:
Seal the tube tightly to prevent leakage or contamination



Step 5
Dispose of the Paper
Tear and flush the paper. If needed, flush again after 15 minutes.

Step 6
Label the Tube and Store
Label with patient's name and date. Put the tube in the clear bag store at room temperature.



Note: To prevent contamination. don't leave the tube open for long, and avoid placing the scoop on surfaces. Ensured that the contents did not spilled, as the liquid inside was crucial for maintaining sample integrity.

A total of 4 stool samples per participant were collected during the study.

5. First Baseline Sample was collected at Baseline day (Day 0) during period I.
6. Second Sample was collected on Day 31 during period I.
7. First Baseline Sample was collected at Baseline day (Day 36) during period II.
8. Second Sample was collected on Day 96 during period II.

For Women
Collected before or after your menstrual period to prevent potential contamination.

After Returning to Normal Routine
If any of the above conditions apply, wait until your gut microbiome stabilizes post-recovery or post-exposure. Then you can proceed with the test.

Preventing Sample Contamination
Before collecting the stool sample, please empty your bladder. Urine or blood In the stool sample lead to inaccurate test results.

Stool sample was shipped at 2°C to 8°C to Centenarians Life Sciences Pvt Ltd. for Microbe Analysis.
• After registration, scheduled a pickup.
• Place the tube in the test box, put it in the return covered and sealed it.

After pickup issues arise, the courier the samples were shipped to below address.
Centenarians Life Sciences Pvt Ltd,
No.7, 1, Haudin Rd, Yellappa Chetty
Layout Sivanchetti Gardens, Halasuru,
Bengaluru, Karnataka 560042

9.5.4.2 Factors affecting drug concentrations and its measurements

- Restrictions regarding water, meals, posture, smoking, chewing tobacco products, alcohol or alcoholic products, xanthine or its derivative containing food or beverages, and grapefruit or its juice are explained under Section 9.1 (Overall Study Design and Plan Description), Section 9.3.1 (Inclusion Criteria) and Section 9.3.2 (Exclusion Criteria).
- Restrictions of prior and concomitant medications are explained in Section 9.3.2 (Exclusion Criteria) and Section 9.4.7 (Prior and Concomitant Therapy).

9.5.4.3 Bioanalysis

Measurement of Total NAD (day -1, day -30, day -36 and day 96)

For NAD+ assay, a kit from Sigma Aldrich., "NAD/NADH Quantitation Kit was used

9.6 DATA QUALITY ASSURANCE

The clinical Investigator/designee ensured that the data entered in the CRFs were as per the current in-house SOPs and in compliance with the study protocol. Quality control personnel checked 100% data of all CRFs as well as pre-study and post-study documents for correctness, completeness, and legibility of the entries. During the study, the Quality Assurance personnel performed the quality audits of involved departments (clinical, pathology, bioanalytical, and biostatistics) and confirmed that the study conduct, bioanalysis, procedures, and the documentation were performed in compliance with the ICH-GCP guidelines, study protocol and the respective in-house SOPs for each activity.

Audit certificate for compliance to SOPs as well as the study protocol ensuring quality, duly signed by the Head of Quality Assurance is provided in **Appendix 16.1.8**.

9.7 STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

9.7.1 Statistical Analysis Plans

(NAD+ STUDY)
Applied the Change from baseline approach on the respective parameters data and presented accordingly.

Appropriate statistical tests were performed on all dependent variables data (i.e., test parameters data) to test significance among the before and after consumption of the coherent water.

P values greater than 0.05 were considered statistically non-significant.

9.7.2 Determination of Sample Size

A total of 10 normal, healthy, adult human subjects were enrolled in the study.

9.8 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

The study was conducted as per the approved study protocol. No changes were made in the conduct of the study or planned analyses.

10.0 STUDY SUBJECTS

10.1 DISPOSITION OF SUBJECTS

A total of 10 normal, healthy, adult, human male subjects were enrolled in the study. The detailed disposition of study subjects is represented in below table and list of discontinued subjects after enrollment is provided in **Appendix 16.2.1**.

Table 6: Disposition of subjects during the study

Enrolled: N = 10		
Activity	**-**	
Period I [Placebo (B)]		
Subjects enrolled	10	
Subjects dosed	10	
Subjects dropout/withdrawn/terminated	00/01[1]/00	
Subjects completing the study	09	
Period II [Test product (A)]		
Subjects enrolled	09	
Subjects dosed	09	
Subjects dropout/withdrawn/terminated	00/01[1]/00	
Subjects completing the study	09	
[1] Subject No.03 withdraw from the study due to personal reason in period I.		
Study completion		
Whole blood samples of number of subjects included in bioanalysis	**09**	
Data of subjects included for statistical analysis	**Test Water (Day 96 - Day 36)**	**08**
	Placebo (Day 31 - Day 0)	**09**
Data of subjects included for safety analysis (by formulation group)	**09**	**10**

10.2 PROTOCOL DEVIATIONS

No protocol deviations reported during the study.

11.0 PHARMACOKINETIC AND DEMOGRAPHIC DATA EVALUATION

11.1 DATA SETS ANALYZED

- **Bioanalysis set:**
  Period I – all (Total 19 samples) Samples of 10 subjects were analyzed.
  Period II – Total 17 Samples of 9 subjects were analyzed. (One sample from subject no. 10 (Day 96) was not analysed due to analytical reason)

- **Statistical set:** Data of 09 subjects were considered for Placebo (Day 31 - Day 0) and Data of 08 subjects were considered for Test Water (Day 96 - Day 36) for statistical assessment.

- **Safety set:** Subjects (N=09 for test product and N=10 for placebo) who received at least one dose of either of the IPs were evaluated for safety.

11.2 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Summary of demographic and baseline data for all the enrolled subjects in the study and subjects considered evaluable for clinical assessment are presented in Section 14.1.

None of the subjects had a history of drug abuse, alcoholism, drug dependence and clinically significant medical history.

Individual baseline demographic data for all the enrolled subjects and subjects considered evaluable for clinical assessment of the study are presented in **Appendix 16.2.4**.

11.3 MEASUREMENTS OF TREATMENT COMPLIANCE

Subjects were instructed to record the details (Quantity, date and time) of water consumption in the subject diary Period I: From day 1 to day 30 and Period II: From day 36 to day 96. Compliance was measured by details of water consumption recorded in subject diary. There was no treatment noncompliance observed in the study.

11.4 PHARMACOKINETIC RESULTS AND TABULATION OF INDIVIDUAL SUBJECT DATA

11.4.1 Statistical Results

Results of Total NAD assessment based on whole blood concentration data of Analemma water are summarized below in Table 7.

Table 7: Mean Summary table of Analemma Water

Mean ±SD (CV %)			
Placebo (C)		Test Product (D)	
Day 0 (N=10)	Day 31 (N=09)	Day 36 (N=09)	Day 96 (N=08)
3044.49 ± 463.27 (15.22)	2559.30 ± 1843.43 (72.03)	8372.76 ± 1026.64 (12.26)	10033.75 ± 1956.77 (19.50)

N- Number of evaluated subjects;

For checking normality we used Shapiro-Wilk Test:

1. **Period 1 (Day 0 vs. Day 31)**:

- Day 0 concentration data (baseline) follows a normal distribution ($p = 0.8777$).
- Day 31 concentration data does not follow a normal distribution ($p < 0.0001$).
- As a result, the baseline-corrected concentration data for the placebo (Day 31 - Day 0) also does not follow a normal distribution ($p < 0.0001$).

- The CV of 72% on Day 31 compared to 15% on Day 0 indicates a substantial increase in relative variability, suggesting higher heterogeneity in the data at Day 31.

2. **Period 2 (Day 36 vs. Day 96)**:

- Both Day 36 (baseline) and Day 96 concentration data follow a normal distribution (p = 0.1116 and p = 0.4408, respectively).
- Therefore, the baseline-corrected concentration data for the test water (Day 96 - Day 36) follows a normal distribution (p = 0.5120).
- For Analemma water (Period 2), the mean increased from Day 36 to Day 96, while the CV values (12.26% and 19.50%) remained relatively low, indicating moderate relative variability and consistent dispersion around the mean.

For Period 1 (Day 0 vs. Day 31) data does not follow normal distribution. So, we used Wilcoxon signed-rank test and For Period 2 (Day 36 vs. Day 96) data follow normal distribution. So, we used Paired T-test and the same results mentioned below:

1. **Placebo (Day 31 - Day 0)**:

- **Wilcoxon signed-rank test**: p = 0.1289 (non-significant).
- This indicates no significant difference between baseline and post-treatment concentration levels for the placebo.

2. **Test Water (Day 96 - Day 36)**:

- **Paired T-test**: p = 0.0200 (significant).
- This suggests a significant improvement or change in NAD+ concentration after consuming Analemma Water compared to the baseline.

For Comparison of Placebo (Day 31 - Day 0) and Test Water (Day 96 - Day 36), we used Wilcoxon Rank-Sum Test.

Comparison between Treatments:

- **Wilcoxon Rank-Sum Test** (non-parametric test for two independent samples):
  - p = 0.0071 (significant).
  - This indicates a statistically significant difference between the baseline-corrected concentrations of the test water (Analemma Water) and placebo. The test water showed a greater effect than the placebo.

11.4.2 Statistical/Analytical Issues

There was no analytical or statistical issue in this study.

11.4.2.1 Adjustments for covariates

No adjustments for Covariates were made.

11.4.2.2 Handling of dropouts or missing data

Disposition of subjects is provided in Section 10.1 (Disposition of Subjects). Data of 09 subjects for Placebo (Day 31 - Day 0) and 08 subjects for Test Water (Day 96 - Day 36) were included in Statistical analysis. There were no missing samples reported during the study.

11.4.2.3 Interim analyses and data monitoring

Not Applicable.

11.4.2.4 Multicentre studies

Not applicable; it was a single centre study.

11.4.2.5 Multiple comparison/multiplicity

Not Applicable.

11.4.2.7 Active-control studies intended to show equivalence

Not Applicable.

11.4.2.8 Examination of subgroups

Not Applicable

11.4.3 Tabulation of Individual Response Data

Not applicable

11.4.4 Drug Dose, Drug Concentration, and Relationships to Response

The subjects received the test product or the placebo Period I: From day 1 to day 30 and Period II: From day 36 to day 96 as per randomization schedule.

11.4.5 Drug-Drug and Drug-Disease Interactions

Not within the scope of the study, hence not applicable.

11.4.6 By-subject Displays

Not applicable

11.4.7 Statistical Conclusions

- Analemma Water showed a significant improvement in NAD+ concentration from baseline to post-treatment ($p = 0.0200$), whereas the placebo showed no significant change ($p = 0.1289$). This suggests that Analemma Water may have a positive effect on NAD+ levels.

- The significant difference between Analemma Water and placebo ($p = 0.0071$) further strengthens the evidence that Analemma Water has a favorable impact compared to placebo drinking water.

This study provides preliminary evidence that Analemma Water may positively affect NAD+ levels.

12.0 SAFETY EVALUATION

12.1 EXTENT OF EXPOSURE

During the study, a total of 10 subjects in period I received single oral dose of Placebo. A total of 09 subjects in period II received single oral dose of Analemma water as per randomization schedule.

The duration of the subject participation was 98 days including washout period of 05 days between consecutive dosing.

12.2 ADVERSE EVENTS (AEs)

12.2.1 Brief Summary of Adverse Events

- A total of 02 adverse events were reported during this study.
- No adverse events were reported during study periods.
- The following two (02) adverse events were reported during post-study safety assessments:
  - ✓ The clinical laboratory report of subject number 04 showed aspartate aminotransferase increased. The increased value was mild in severity and judged as unlikely related to the study drug. Subject was called for repeat safety laboratory test and followed up until resolution of AEs.
  - ✓ The clinical laboratory report of subject number 05 showed eosinophil count increased. The increased value was moderate in severity and judged as unlikely to be related to study drug. This subject was called repeatedly for post study follow-up, but he was unable to report to the clinical facility due to personal reasons, however he informed by a telephone call that he did not have any health-related issues.
- There were no SAEs reported during the study.

12.2.2 Display of Adverse Events

Number of AEs experienced by the subjects is presented in below table. Details of individual subject AEs are presented in Section 14.3, (Safety Data Summary Tables) (Table 10).

Table 8: Number of AEs experienced by the subjects

Body system/Adverse event	Test product (N=09)	Placebo (N=10)
Investigation[1]		
Aspartate aminotransferase increased	01	
Eosinophil count increased	01	
Total	**01**	

[1]Post-study clinical laboratory assessment was performed after completion of the study, hence not attributed to any of the treatments.

12.2.3 Analysis of Adverse Events

A total of 02 adverse events were reported during this study. Out of which, no AEs were related to the test product and Placebo; and 02 AEs were reported during post study safety assessments, which could not be attributed to any of the study periods or treatments.

12.2.4 Listing of Adverse Events by Subject

A listing of AEs is provided in Section 14.3 and **Appendix 16.2.7.**

12.3 DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

No deaths, no other serious adverse events or other significant adverse events were reported during the study.

12.3.1 Listing of Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

12.3.1.1 Deaths

None.

12.3.1.2 Other Serious Adverse Events

None.

12.3.1.3 Other Significant Adverse Events

None.

12.3.2 Narratives of Deaths, Other Serious Adverse Events, and Certain Other Significant Adverse Events

Not applicable.

12.3.3 Analysis and Discussion of Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

Not applicable.

12.4 CLINICAL LABORATORY EVALUATION

12.4.1 Listing of Individual Laboratory Measurements by Subject

Individual laboratory measurement by subjects is provided in individual subject Case Report Form**.**

12.4.2 Clinical Evaluation of Each Laboratory Parameter

Individual laboratory parameters were evaluated against acceptable ranges mentioned in laboratory report.

12.4.2.1 Laboratory Values Over Time

Not applicable.

12.4.2.2 Individual Subject Changes

Not applicable.

12.4.2.3 Individual Clinically Significant Abnormalities

Clinically significant laboratory abnormalities were reported as AEs and are presented under Section 14.3, (Safety Data Summary Tables) Table 10.

12.5 VITAL SIGNS, PHYSICAL FINDINGS, AND OTHER OBSERVATIONS RELATED TO SAFETY

No clinically significant vital signs, physical findings, and other observations were reported in this study.

12.6 SAFETY CONCLUSIONS

There were no SAEs observed during the study and no AEs with either the test product (Analemma Water) and the Placebo drinking water. Based on the safety results explained above, both the test product (Analemma Water) and the Placebo drinking water were well tolerated during the study.

13.0 DISCUSSION AND OVERALL CONCLUSIONS

- Change in Mean within the Placebo (Period 1) i.e. From Day 0 to Day 31: 2559.30 - 3044.49 = -485.19 it means that the mean is decreased by 485.19 units i.e. 19% lower in Period 1. Similarly, for Change in Mean within the Test Product (Period 2) i.e. From Day 36 to Day 96: 10033.75 - 8372.76 = 1660.99 it means that the mean increased by 1660.99 units. i.e. 20% higher in Period 2.
- The CV of 72% on Day 31 compared to 15% on Day 0 indicates a substantial increase in relative variability, suggesting higher heterogeneity in the data at Day 31. Similarly, For Analemma water (Period 2), the mean increased from Day 36 to Day 96, while the CV values (12.26% and 19.50%) remained relatively low, indicating moderate relative variability and consistent dispersion around the mean.
- Analemma Water showed a significant improvement in NAD+ concentration from baseline to post-treatment ($p = 0.0200$), whereas the placebo showed no significant change ($p = 0.1289$). This suggests that Analemma Water may have a positive effect on NAD+ levels.
- The significant difference between Analemma Water and placebo ($p = 0.0071$) further strengthens the evidence that Analemma Water has a favorable impact compared to placebo drinking water.
- This study provides preliminary evidence that Analemma Water may positively affect NAD+ levels.
- There were no safety concerns either with test product or reference product.

14.0 TABLES, FIGURES AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT

14.1 DEMOGRAPHIC DATA SUMMARY AND TABLES

Summary of demographic and baseline data for all the enrolled subjects in the study and subjects considered evaluable for clinical assessment of the study are presented in below tables.

Table 9: Demographic and baseline data of all the enrolled subjects (N=10)

N = 10				
Parameter	**Age (yrs)**	**Weight (kg)**	**Height (cm)**	**BMI (kg/m^2)**
Mean	30.00	67.15	166.08	24.42
SD	4.62	9.15	9.07	3.36
Median	28.50	67.65	167.70	24.96
Min	25.00	53.40	153.00	18.59
Max	38.00	80.00	179.30	29.54
% CV	15.40	13.63	5.46	13.76
Sex				
Male	10 (100.00%)			
Female	00			
Race				
Asian	10 (100%)			
Other	00			

Table 10: Demographic and baseline data of all the evaluable subjects (N=090

N = 09				
Parameter	**Age (yrs)**	**Weight (kg)**	**Height (cm)**	**BMI (kg/m^2)**
Mean	30.56	65.72	165.48	24.11
SD	4.53	8.44	9.40	3.41
Median	30.00	62.00	165.90	24.92
Min	26.00	53.40	153.00	18.59
Max	38.00	75.00	179.30	29.54
% CV	14.83	12.85	5.68	14.15
Sex				
Male	10 (100.00%)			
Female	00			
Race				
Asian	10 (100%)			
Other	00			

14.3 SAFETY DATA SUMMARY TABLES

Total of 02 adverse events were reported during post study safety assessment described as below:

Table 11: Post-study safety laboratory test results with significant values

Subject no.	Parameter /AE	Baseline Laboratory Value	Post Study Laboratory Values	Acceptable range	Follow up Values	Repeated Follow up	Expectedness (Yes/No)	Severity (Grade)	Causality	Outcome	Start Date	End Date	Start Time	End Time
04	Aspartate Aminotransferase Increased	38.14 U/L	108.05 U/L	0-91U/L	46.42 U/L	No	No	Grade 1 - Mild	Unlikely	Resolved	12/09/24	18/09/24	16:09	12:19
05	Eosinophil Count Increased	14%	21.10%	01-10 %	Lost to follow up	NA	NO	Grade 2 - Moderate	unlikely	Lost to Follow up	12/09/24	NA	15:58	NA

Table 12: Concomitant Medication for management of AE

Sub No.	Period	Generic name	Brand name	Start date	Start time	End date	End time	Frequency
05	II	Albendazole 400 mg	Tablet-Zentel	22/09/24	22:15	22/09/24	22:15	1 tablet single dose at bed time
		Diethylcarbamazine 100 mg	Tablet-Hetrazan	22/09/24	22:00	29/09/24	14:00	1 tablet three times a day for 7 days

15.0 REFERENCE LIST

1. Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects, World Medical Association – 64th WMA General Assembly, Fortaleza, Brazil, October 2013.
2. Integrated Addendum to ICH E6 (R1): Guideline For Good Clinical Practice E6 (R2), Current Step 4 version dated 9 November 2016.
3. New Drugs and Clinical Trials (Amendment) Rules, 2022 [Gazette notification G.S.R.227 (E) dated 19.03.2019, G.S.R.605 (E), dated 31.08.2021 G.S.R 778 (E) dated 14 Oct 2022], Ministry of Health and Family Welfare, Government of India.
4. Guidelines for Bioavailability and Bioequivalence Studies, Central Drug Standard Control Organization, Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India, New Delhi, (March 2005).
5. National Ethical Guidelines for Biomedical and Health Research Involving Human Participants, Indian Council of Medical Research, 2017.
6. https://www.who-umc.org/media/164200/who-umc-causality-assessment_new-logo.pdf (The use of the WHO-UMC system for standardised case causality assessment, 2018-04-06).
7. http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi (A Healthy lifestyle – WHO recommendations 6 May 2010).
8. Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, Published: November 27, 2017, U.S. Department of Health and Human Services.

16.0 APPENDICES

16.1 STUDY INFORMATION

16.1.1 Protocol and Protocol Amendments

16.1.2 Sample Case Report Form

16.1.3 List of IECs or IRBs (plus the name of the committee Chair if required by the regulatory authority) - Representative written information for subject and sample consent forms

16.1.4 List and description of investigators and other important participants in the study, including brief (1 page) CVs or equivalent summaries of training and experience relevant to the performance of the clinical study

16.1.5 Signatures of principal or coordinating investigator(s) or sponsor's responsible medical officer, depending on the regulatory authority's requirement

16.1.6 Listing of subjects receiving test drug(s)/investigational product(s) from specific batches, where more than one batch was used

16.1.7 Randomization scheme and codes (subject identification and treatment assigned)

16.1.8 Audit certificates

16.1.9 Documentation of statistical methods

16.1.10 Documentation of inter-laboratory standardization methods and quality assurance procedures if used

16.1.11 Publications based on the study

16.1.12 Important publications referenced in the report

16.2 SUBJECT DATA LISTINGS

16.2.1 Discontinued subjects

16.2.2 Protocol Deviations

16.2.3 Subjects excluded from the efficacy analyses

16.2.4 Demographic Data

16.2.5 Compliance and /or Drug Concentration Data

16.2.6 Individual efficacy response data (16.2.6.1 and 16.2.6.2)

16.2.7 Adverse event listings (each subject)

16.2.8 Summary Report for Analytical Method Development for Estimation of NAD/NADH in Whole Blood Samples By Colorimetric Method