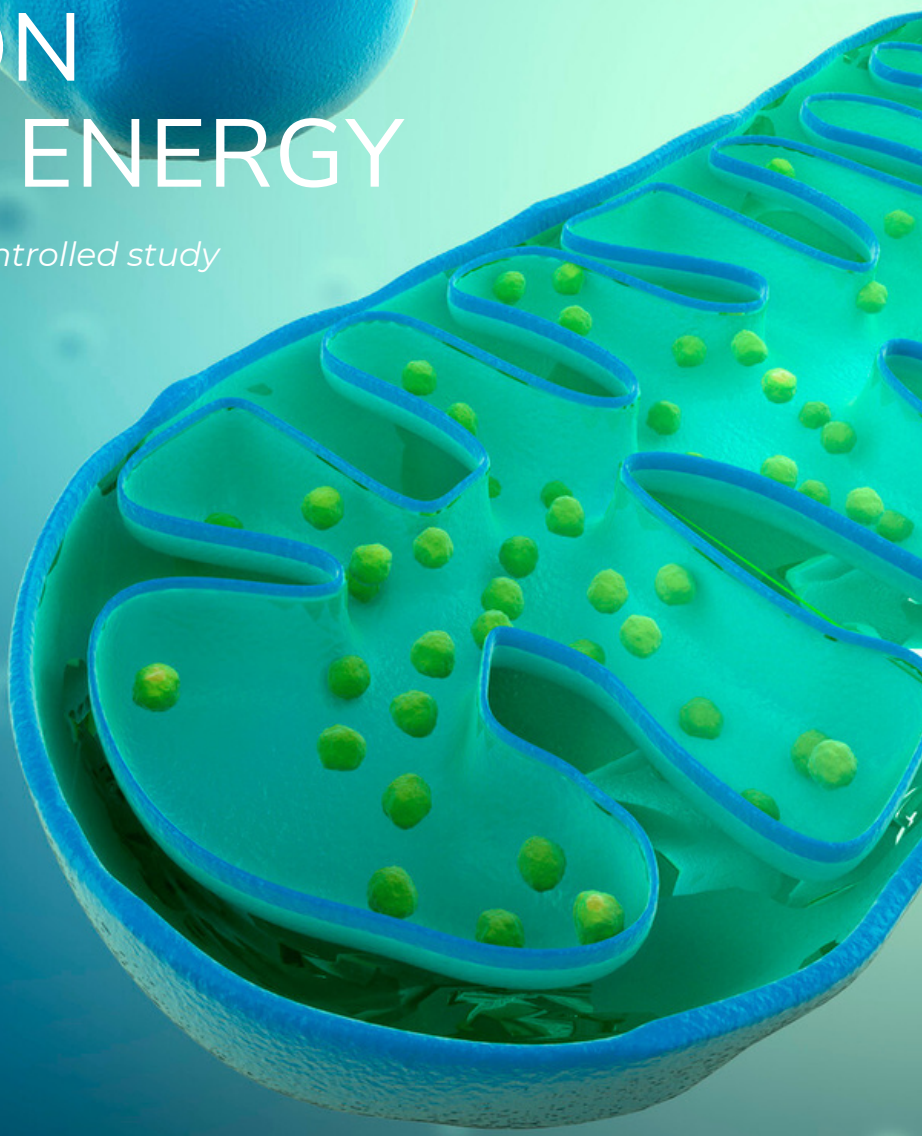
ANALEMMA WATER
RESEARCH REPORT

JANUARY 2023

EFFECTS ON CELLULAR ENERGY

2022 double-blind placebo-controlled study



ANALEMMA

THE ARCHITECT OF LIFE

The study of ATP levels in humans (2022)

The aim of the study was to measure the effect of prolonged Aȟalemma water consumption on blood ATP levels of human participants.

Adenosine triphosphate (ATP) is commonly referred to as the "energy currency" of the cell. Its structure is simple - it consists of a base, a sugar and three serially linked phosphate groups. The energy of ATP is stored in the bond between its second and third phosphate group. Whenever this bond is broken (hydrolyzed), energy is released.

The majority of cellular ATP is synthesized in the mitochondria and then exchanged as fuel for other biochemical reactions. ATP synthesis and hydrolysis are in a constant cycle and both need to be maintained for proper cellular functioning. Inadequate production of ATP adversely affects numerous cellular processes and can have deleterious effects on human health.

WHAT IS ATP USED FOR?

ATP is utilized in a number of biological processes, such as **DNA and RNA synthesis, muscle contraction, neuronal impulse propagation** and many others. The role of ATP in muscle contraction is multifold - ATP is necessary for generating force and maintaining ion transport across membranes, making it essential for everyday muscle functioning.

Even more demanding is the utilization of ATP in the brain. A single neuron hydrolyzes nearly one billion ATP molecules for a single repolarization event, which happens every time it needs to send an impulse to a neighboring cell. Not surprisingly, the brain is the highest consumer of ATP, spending around 25% of total available energy in the body.

RESEARCH REPORT

JANUARY 2023

Effects of Aṅalemma on human ATP levels (2022)

EXPERIMENTAL DESIGN

OBJECTIVE: To assess the effect of prolonged Aṅalemma water consumption on ATP levels in the blood of healthy adult humans.

PARTICIPANTS: 50 healthy adult human subjects, aged 18-60, BMI 18.50-29.99 kg/m^2.

STUDY DESIGN: The study was designed as a **double blind, placebo-controlled, randomized, parallel group clinical study**. Participants were randomly divided into three groups and given adequate amounts of Aṅalemma Water (n=13), Test Water 2 (n=12) or non-treated water (Placebo Control, n=25). The setup was double blind, with neither the participants nor the clinical investigators aware of how the subjects were distributed into groups. All participants were instructed to consume at least 1.5 L of the given water for 60 days, with regular compliance assessment check-ups. ATP levels of all participants were measured in whole blood samples obtained prior to treatment (Day -1) and on the last day of treatment (Day 60). Relative ATP levels were detected using a standardized kit based on firefly luciferase bioluminescence (CellTiter-Glo Luminescent Cell Viability Assay, Promega).

DATA ANALYSIS: The change in ATP levels between Day -1 and Day 60 was calculated for all groups. Statistical difference between the mean values obtained in the Aṅalemma Water group and the Placebo Control group was analyzed by one-way analysis of variance (ANOVA).

RESULTS

The change in ATP levels in the Aṅalemma group was 26.84 ± 24.90, while the change in ATP levels in the Placebo Control group was 7.32 ± 26.65. The difference between the means was significant. Therefore, this study indicates that **consuming Aṅalemma water for 60 days significantly increases blood ATP levels in humans**.

INSTITUTION

Raptim Research Pvt. Ltd. (Navi Mumbai, India)

RAPTIM RESEARCH LTD.
A CONTRACT RESEARCH ORGANIZATION

CLINICAL INVESTIGATOR: Dr. Yashvant Khaire
Clinical Co-Investigator: Dr. Nandkishor Gameti, M.B.B.S., Dr. Raviraj Jagdhani, M.B.B.S. M.D. Pharmacology
Bioanalytical Investigator: Dr. Milind Bagul, M. Pharm, Ph.D.
Biostatistician: Veerababu Yegi, M.Sc. Statistics
Head of Quality Assurance: Usha Ramakrishnan, B. Pharm

RESEARCH REPORT
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Effects of Aṅalemma on human ATP levels (2022)

1. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The study was performed in collaboration with Raptim Research Pvt. Ltd., (Navi Mumbai, India). The administrative structure of the study is described in **Table 1**.

Table 1. Administrative structure of the study

<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="5">Raptim Research Pvt. Ltd.,
Clinical Pharmacology Unit (Clinic and Pathology): A-226;
Screening Facility (PAP-213, PAP-A-218 and PAP-A-219);
Bioanalytical and Biostatistical Unit: A-242;
T.T.C., Industrial Area, Mahape M.I.D.C., Navi Mumbai – 400 710, India.
Tel. No.: +912227781889: Ext. No. 112 (Clinic); 120 (Pathology); 159 (Bioanalytical and Statistical); Fax No.: +912227781884</td></tr>
<tr><td>Clinical Co-Investigator:
Dr. Nandkishor Gameti, M.B.B.S.
Dr. Raviraj Jagdhani, M.B.B.S. M.D. Pharmacology</td></tr>
<tr><td>Bioanalytical Investigator:
Dr. Milind Bagul, M. Pharm, Ph.D.</td></tr>
<tr><td>Biostatistician:
Mr. Veerababu Yegi, M.Sc. Statistics</td></tr>
<tr><td>Head of Quality Assurance:
Mrs. Usha Ramakrishnan, B. Pharm.</td></tr>
<tr><th colspan="2">Independent Ethics Committee</th></tr>
<tr><td colspan="2">HumanCare Independent Ethics Committee Shop. No. 16, Lodha Elite, Near Nilje Railway Station, Lodha Heaven, Dombivali (East), Thane 421204, India.
Tel. No.: +91 77383 60789
Email: humancareethics@gmail.com</td></tr>
<tr><th colspan="2">Sponsor’s Representative</th></tr>
<tr><td colspan="2">Madhusudan Rajagopalan (Director)
Water and Light Applications India Private Limited 142, 6A, Kalpataru Estate. JV Link Road Andheri East Mumbai 400 093</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.
Tel. No.: +912227781889, Ext. no.: 120
Fax No.: +912227781884</td></tr>
</table>

2. ETHICS

2.1 INDEPENDENT ETHICS COMMITTEE (IEC)

The study protocol version 00, dated 07/07/22, Subject Information Sheet and Informed Consent Form (SIS-ICF) version 00 (dated 08/07/22; English, Hindi, and Marathi), Investigational Product (IP) Information and other protocol related documents were reviewed and approved by HumanCare Independent Ethics Committee in the meeting held on 17/07/22.
The study protocol amendment version 00, dated 18/07/22 to protocol version 00, dated 07/07/22, and other protocol related documents were reviewed and approved by HumanCare Independent Ethics Committee in the meeting held on 19/07/22.

2.2 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, Good Clinical Practice (International Council for Harmonization – E6 (R2) Guidelines, Current Step 4 Version, dated 9 Nov 2016)2, New Drugs and Clinical Trials (Amendment) Rules, 2021 [Gazette notification G.S.R.227 (E), dated 19.03.2019 & G.S.R.605 (E), dated 31.08.2021] , Ministry of Health and Family Welfare, Government of India3, CDSCO Guidelines for Bioavailability & Bioequivalence Studies Mar 20054, Indian Council of Medical Research (Ethical Guidelines for Biomedical Research on Human Participants, 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 pharmacokinetic (PK) parameter and assessment of safety in the present study.

2.3 SUBJECT INFORMATION AND CONSENT

All the subjects screened for the study received information both verbally and in written form in English 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 English 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. A copy of the signed and dated study specific SIS-ICF was provided to the individual subject.

3. OVERALL STUDY DESIGN

This was a double blind, placebo controlled, randomized, parallel group clinical study conducted in 50 normal, healthy, adult, human subjects. Subjects were randomized into two treatment sequence groups. The study was conducted in following phases based on the activities performed.

3.1 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), well-being, 12-lead ECG recording, laboratory investigations (hematology, biochemistry, serology and urine analysis), urine pregnancy test, gynecological history (for female subjects) and evaluation of inclusion and exclusion criteria. Subjects were given awareness instructions for corona virus disease, consent was taken and assessment was performed for COVID-19.

3.2 TREATMENT PHASE

The following activities were performed from the day of check-in until post-study safety assessments.

3.2.1 Baseline Activities (Day -1)

One day prior to consumption of Aὴalemma Water, Test Water 2 or Placebo drinking water multiple activities were performed, including biometric identification, obtaining study specific written informed consent, 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 samples were obtained at Baseline (Day -1) for ATP level analysis.

Healthy subjects were randomized to receive either Aὴalemma Water, Test Water 2 or Placebo for 90 consecutive days.

3.2.2 Treatment

During the treatment phase (Day 1 - Day 90), subjects were instructed to consume a daily dose of at least 1.5 L of the water that was supplied to them according to the randomization schedule

3.2.3 Compliance checks and safety assessments

Water consumption compliance and safety were assessed during Compliance Check Visits**.** The safety of subjects was assessed by monitoring for occurrence of any adverse effects 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. All subjects visited the facility every 10 days up to day 80 for water consumption compliance check (i.e. on Day 10, Day 20, day 30, day 40, Day 50, Day 70, and Day 80; **Table 2**). Subjects were allowed to visit the facility ±2 days from above scheduled days. A post-study safety assessment was conducted at the end of the study (Day 91).

Table 2. Study activities organized by phase, day and visit number.

Phase	Day	Visit No.	Activity
Baseline Day	Day -01	01	Blood sample collection for ATP level analysis.
Compliance Check	Day 10	02	Water consumption compliance check
	Day 20	03	Water consumption compliance check
	Day 30	04	Water consumption compliance check
	Day 40	05	Water consumption compliance check
	Day 50	06	Water consumption compliance check
ATP levels of whole blood	Day 60	07	Blood sample collection for ATP levels of whole blood and Water consumption compliance check
Compliance Check	Day 70	08	Water consumption compliance check
	Day 80	09	Water consumption compliance check
Post Study Safety Assessment	Day 91	10	Post study safety assessment

4. 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.

4.1 INCLUSION CRITERIA

A subject who 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 18-60 years (both inclusive) of age
- Body mass index in the range of 18.50 – 29.99 kg/m2 (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

4.2 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
- Requiring medication for any ailment having enzyme-modifying activity within one month prior to baseline day visit and throughout the study
- Use of any depot injection or an implant of any drug within 3 months prior to baseline day visit 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 and throughout the study
- Vaccinated 7 days prior to baseline day visit and willing to get vaccinated throughout 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

4.3 REMOVAL OF SUBJECTS FROM THE STUDY

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

- 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

Summary of demographic and baseline data for all the enrolled subjects in the study and subjects considered eligible for clinical assessment are presented in **Table 3**.

Table 3: Demographic and baseline data of all subjects enrolled in the study (n=50)

Parameter	Age (yrs)	Weight (kg)	Height (cm)	BMI (kg/m2)
Mean	30.22	67.34	163.84	25.03
SD	6.91	11.00	8.96	3.14
Median	28.50	67.90	164.05	25.20
Min	21.00	46.00	144.00	18.57
Max	51.00	92.00	180.00	29.81
% CV	22.87	16.33	5.47	12.56
Sex				
Male	36 (72%)			
Female	14 (28%)			
Race				
Asian	50 (100%)			

5. RANDOMIZATION AND BLINDING

5.1 SUBJECT RANDOMIZATION

As per the study design, (A and B) 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 numbers assigned to subjects were as follows:

Water-1 dosed Subjects: 2, 3, 6, 7, 9, 11, 13, 16, 18, 19, 21, 23 and 25.

Water-2 dosed Subjects: 28, 29, 31, 34, 36, 38, 40, 42, 44, 46, 48 and 49.

Placebo dosed Subjects: 1, 4, 5, 8, 10, 12, 14, 15, 17, 20, 22, 24, 26, 27, 30, 32, 33, 35, 37, 39, 41, 43, 45, 47 and 50.

5.2 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 Pharmacist, the assistant pharmacist who are responsible for dispensing of investigational products and the QA auditor monitoring the dispensing activity had access to the randomization schedule and they were not be involved in any other study related activities until completion of the analysis.
The pharmacist assigned 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 placebo treatment. The treatment code assigned for test and placebo 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 after completion of clinical and after completion of ATP level analysis of the study for statistical analysis.
For code breaking procedure (in case of occurrence of any SAE or in any emergency condition citing safety concern of the subject) individual envelope containing the label with the details of subject number and the randomization sequence of the subject was prepared by the Pharmacist prior to baseline day visit. These labels were verified with the randomization schedule by the assistant pharmacist and the QA auditor. The sealed envelopes with subject number written on were kept in a secure place in Pharmacy or with Clinical Investigator.

6. 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 **Table 4**. 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.

The IPs were delivered in form of tubes, producing either Aṅalemma Water, Test Water 2 or Placebo (i.e. not changing the water in any way). All the unused IPs were returned to the pharmacy and stored in the same formulation cabinet with that of the undispensed IPs.

6.1 PREPARATION OF WATER

Participants were instructed to use their designated Water Tube exclusively on drinking water safe for human consumption: water of good quality that has already been filtered and purified and that is free from chemical and biological pollution.

Table 4. Investigational product details.

Parameter	Test product (A)	Reference product (B)
Product name	Aṅalemma Water, Test Water 2	Placebo
Manufacturer	Water and Light Applications India Private Limited	Water and Light Applications India Private Limited

7. TREATMENTS

7.1 TREATMENT ADMINISTRATION

From Day 1 to Day 90, the technology to produce Aȟalemma Water, Test Water 2 or Placebo drinking water was provided to all subjects according to the randomization schedule. Subjects were instructed to consume a minimum of 1.5 L of water daily. Subjects were instructed to record the details (quantity, date and time) of water consumption in the subject diary from day 1 to day 90.Details of water treatment administration are provided in **Table 5**.

Table 5. Treatment administration details.

Parameter	Test Product (A)	Placebo (B)
Dosage form	Water (Aȟalemma Water and Test Water 2)	Water
Route	Oral	Oral
Dose and Mode of administration	Technology to produce Aȟalemma Water or Test Water 2 was provided to subjects, with instruction to consume a minimum of 1.5 L of water daily from day 1 to day 90 at ambient temperature	Technology to produce placebo drinking water was provided to subjects, with instruction to consume a minimum of 1.5 L of water daily from day 1 to day 90 at ambient temperature
Baseline Day Date (Day 01, Group 1)	22/07/22	
Treatment Start Date (Day 01, Group 2)	24/07/22	
Post Study Date (Day 91, Group 1)	20/10/22	
Post Study Date (Day 91, Group 2)	22/10/22	

7.1.1 Restrictions of fluid and other substances

Consumption of normal drinking water (i.e. water not supplied through the study) was restricted during the duration of the study. 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.

8. SAMPLING, MEASUREMENTS AND ANALYSIS

8.1 BLOOD SAMPLE COLLECTION AND PROCESSING

Blood samples (15.0 mL) were collected during Baseline day -1 (Visit 01) and during post study safety assessment on Day 60 (Visit 7). Blood samples were collected in pre-labeled vacutainers containing K3EDTA as an anticoagulant. A total of two blood samples were collected for ATP level analysis. After sample collection, vacutainers were stored in the refrigerator (at 2°C to 8°C). After collection of samples from all subjects, samples were shipped at 2°C to 8°C to the bioanalytical facility of Raptim Research Pvt. Ltd. (A-242).

8.1.1 Blood loss

Total blood loss for a subject during the study did not exceed 55.0 mL (for male subjects) or 57.0 mL (for female subjects).

8.2 BIOANALYSIS

8.2.1 ATP measurement

Blood ATP levels were measured by the firefly bioluminescence assay kit (AMERIC-ATP kit; Wako Pure Chemical Industries, Osaka, Japan) according to the protocol supplied by the manufacturer. ATP levels were measured from whole blood samples of all participants obtained on Day -1 (baseline) and on Day 60 (after water consumption).

8.3 STATISTICAL ANALYSIS

Statistical analysis was performed using SAS® Version 9.4 or higher (SAS Institute Inc., USA). The individual and descriptive summary statistics was performed for ATP levels of whole blood before consumption of water (Day -1) and after consumption of water (Day 60). Measurements obtained on Day 60 were compared with Baseline measurements (Day -1) for each participant and the percentage of change was calculated (**Table 6**). A one-way analysis of variance (ANOVA) was performed on ATP levels data to test significance of change values (before and after consumption of water) between Aṅalemma Water group and Placebo group (**Table 7**). Mean change values in ATP levels calculated for Test Water 2 group are presented in **Table 8**. Analysis of variance was not performed for the Test Water 2 group relative to Placebo.

8.4 RESULTS

The mean change in ATP levels calculated for the Aṅalemma Water group was 26.8414705 ± 24.9047948 (mean ± standard deviation). The mean change in ATP levels calculated for the Placebo group was 7.3233438 ± 26.6464522 (mean ± standard deviation). According to ANOVA, the change in ATP levels measured before and after consumption of water was significantly higher in the group consuming Aṅalemma Water (Water-1), than Placebo, with the probability value of 0.0352 (**Table 7**).

Table 6. ATP levels were measured before (Day -1) and after consumption of water (Day 60). Mean change in ATP levels was calculated for Aȟalemma Water group (n=13) and Placebo group (n=25). Mean values and standard deviations (SD) are listed.

Group	Aȟalemma Water (n=13)	Placebo (n=25)
Change in ATP level (mean ± SD)	26.8414705 ± 24.9047948	7.3233438 ± 26.6464522

Table 7. Analysis of variance (ANOVA) performed for values of change in ATP levels (before and after consumption of water) between Aȟalemma Water group and Placebo group. The main ANOVA parameters (sum of squares, mean square, F-value and p-value) are presented.

Sum of Squares	**Mean square**	**F Value**	**p-value**	**Result**
3258.187159	3258.187159	4.79	0.0352	Significant

f P ≤ 0.05, the result is Significant;
If P > 0.05, the result is Non-Significant.

Table 8. ATP levels were measured before (Day -1) and after consumption of water (Day 60). Mean change in ATP levels was calculated for Test Water 2 group (n=12) and Placebo group (n=25). Mean values and standard deviations (SD) are listed.

Group	Test Water 2 (n=12)	Placebo (n=25)
Change in ATP level (mean ± SD)	5.87461351 ± 19.0046343	7.3233438 ± 26.6464522

9. POST-STUDY SAFETY ASSESSMENT

Post-study safety evaluations were performed on day 91. 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) were performed. The schedule of study events (**Table 9**) and a list of laboratory tests performed (**Table 10**) as part of the screening and post-study safety examinations are presented below.

Table 9. The complete schedule of study events

Procedure	Screening (within 21 days prior to baseline day -1)	Treatment period		Post Study (Day 91) of a subject
		Baseline Day (Day -1)	Compliance Check Day 10, Day 20, Day 30, Day 40, Day 50, Day 60, Day 70, and Day 80	
Screening consent form	X			
Study specific informed consent		X[#]		
Demographics	X			
Medical and medication History	X	X		
Physical examination	X	X		
Vital signs	X	X	X	
Well-being		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		
Compliance Check		X[$$]	X	
Urine screen for drugs of abuse		X		
Urine alcohol test		X		
Water Consumption[%]			X	
Blood samples		X*	X*	X
Safety monitoring		X	X	X

[#]Only on baseline day -1
* Blood samples were collected for ATP levels of whole blood (Day -1 and Day 60)
[$$]Compliance check was performed on Day 10, Day 20, Day 30, Day 40, Day 50, Day 60, Day 70, and Day 80
[%] From Day 1 to Day 90.

Table 10. Laboratory tests performed during the study

Hematology	Biochemistry	#Urine analysis	
		Routine	Microscopy
• Erythrocyte Count • Hemoglobin • WBC Count • Platelet Count • Neutrophils • Eosinophils • Lymphocytes • Basophils • Monocytes	• Serum Alkaline Phosphatase • Serum Creatinine • Serum SGOT/AST • Serum SGPT/ALT • Serum Uric Acid • Serum Blood Urea Nitrogen • Plasma Glucose (Random) • Serum Bilirubin - Total	• Color • Appearance • Reaction pH • Protein (Albumin) • Ketone bodies • Sugar (Glucose) • Occult Blood • Urobilinogen	• Red Blood Cells • Pus Cells • Epithelial Cells • Casts • Crystals • Bacteria
#Serology		*Urine Examination For Drug Abuse	
• HIV antibodies (I &II) • HBsAg • HCV		• Benzodiazepines (BZD) • Barbiturates (BAR) • Tetrahydrocannabinol (THC)	• Opiate (OPI) • Cocaine (COC) • Amphetamine (AMP)

*Activities at baseline (Day -1).
#At screening only

9.1 SAFETY ASSESSMENT 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.

9.2 SAFETY ASSESSMENT RESULTS

- No adverse events were reported during study periods.
- No adverse events were reported during post-study safety assessments.
- There were no SAEs reported during the study.

10. DATASETS

- Bioanalysis dataset: Samples of all 50 subjects were analyzed for ATP levels.
- Overall statistics dataset: As per the study protocol, data (ATP levels) of 50 subjects completing study period was considered for statistical analysis.
- Assessment dataset: Data of 38 subjects for Water-1 (13 subjects) and Placebo (25 subjects) were considered for statistical assessment. However, data of 37 subjects for Water-2 (12 subjects) and Placebo (25 subjects) were evaluated and reported as additional information.
- Safety dataset: Subjects (N=25 for test product and N=25 for placebo) who received at least one dose of either of the IPs were evaluated for safety.

11. 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.

12. REFERENCES

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This document is an abbreviated version of the original clinical report obtained by Raptim Research Pvt. Ltd. following the completion of the study. The clinical report is available upon request.

RESEARCH REPORT

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