1. CONSORT CHECKLIST

Section/topic	No	CONSORT 2025 checklist item description	Reported on page no.
Title and abstract			
Title and structured abstract	1a	Identification as a randomised trial	2
	1b	Structured summary of the trial design, methods, results, and conclusions	2-3
Open science			
Trial registration	2	Name of trial registry, identifying number (with URL) and date of registration	5
Protocol and statistical analysis plan	3	Where the trial protocol and statistical analysis plan can be accessed	Supplementary files
Data sharing	4	Where and how the individual de-identified participant data (including data dictionary), statistical code and any other materials can be accessed	Unique identifiers were assigned to the partcipants.
Funding and conflicts of interest	5a	Sources of funding and other support (eg, supply of drugs), and role of funders in the design, conduct, analysis and reporting of the trial	18
	5b	Financial and other conflicts of interest of the manuscript authors	18
Introduction			
Background and rationale	6	Scientific background and rationale	3-4
Objectives	7	Specific objectives related to benefits and harms	9-10
Methods			
Patient and public involvement	8	Details of patient or public involvement in the design, conduct and reporting of the trial	5
Trial design	9	Description of trial design including type of trial (eg, parallel group, crossover), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Changes to trial protocol	10	Important changes to the trial after it commenced including any outcomes or analyses that were not prespecified, with reason	-
Trial setting	11	Settings (eg, community, hospital) and locations (eg, countries, sites) where the trial was conducted	6-9
Eligibility criteria	12a	Eligibility criteria for participants	N/A
	12b	If applicable, eligibility criteria for sites and for individuals delivering the interventions (eg, surgeons, physiotherapists)	8
Intervention and comparator	13	Intervention and comparator with sufficient details to allow replication. If relevant, where additional materials describing the intervention and comparator (eg, intervention manual) can be accessed	9-10
Outcomes	14	Prespecified primary and secondary outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome	10
Harms	15	How harms were defined and assessed (eg, systematically, non-systematically)	11
Sample size	16a	How sample size was determined, including all assumptions supporting the sample size calculation	10

	16b	Explanation of any interim analyses and stopping guidelines	8
Randomisation:			
Sequence generation	17a	Who generated the random allocation sequence and the method used	8
	17b	Type of randomisation and details of any restriction (eg, stratification, blocking and block size)	8
			8
Allocation concealment mechanism	18	Mechanism used to implement the random allocation sequence (eg, central computer/telephone; sequentially numbered, opaque, sealed containers), describing any steps to conceal the sequence until interventions were assigned	8
Implementation	19	Whether the personnel who enrolled and those who assigned participants to the interventions had access to the random allocation sequence	8
Blinding	20a	Who was blinded after assignment to interventions (eg, participants, care providers, outcome assessors, data analysts)	8
	20b	If blinded, how blinding was achieved and description of the similarity of interventions	10
Statistical methods	21a	Statistical methods used to compare groups for primary and secondary outcomes, including harms	7-8
	21b	Definition of who is included in each analysis (eg, all randomised participants), and in which group	8
	21c	How missing data were handled in the analysis	-
	21d	Methods for any additional analyses (eg, subgroup and sensitivity analyses), distinguishing prespecified from post hoc	8
Results			
Participant flow, including flow diagram	22a	For each group, the numbers of participants who were randomly assigned, received intended intervention, and were analysed for the primary outcome	9
	22b	For each group, losses and exclusions after randomisation, together with reasons	9
Recruitment	23a	Dates defining the periods of recruitment and follow-up for outcomes of benefits and harms	-
	23b	If relevant, why the trial ended or was stopped	-
Intervention and comparator delivery	24a	Intervention and comparator as they were actually administered (eg, where appropriate, who delivered the intervention/comparator, how participants adhered, whether they were delivered as intended (fidelity))	6
	24b	Concomitant care received during the trial for each group	-
Baseline data	25	A table showing baseline demographic and clinical characteristics for each group	11
Numbers analysed, outcomes and estimation	26	For each primary and secondary outcome, by group: ● the number of participants included in the analysis ● the number of participants with available data at the outcome time point ● result for each group, and the estimated effect size and its precision (such as 95% confidence interval) ● for binary outcomes, presentation of both absolute and relative effect size	9-10
Harms	27	All harms or unintended events in each group	14
Ancillary analyses	28	Any other analyses performed, including subgroup and sensitivity analyses, distinguishing pre-specified from post hoc	Added as supplementary material
Discussion			11-14
Interpretation	29	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	17
Limitations	30	Trial limitations, addressing sources of potential bias, imprecision, generalisability, and, if relevant, multiplicity of analyses	

Citation: Hopewell S, Chan AW, Collins GS, Hróbjartsson A, Moher D, Schulz KF, et al. CONSORT 2025 Statement: updated guideline for reporting randomised trials. BMJ. 2025; 388:e081123. https://dx.doi.org/10.1136/bmj-2024-081123

*We strongly recommend reading this statement in conjunction with the CONSORT 2025 Explanation and Elaboration and/or the CONSORT 2025 Expanded Checklist for important clarifications on all the items. We also recommend reading relevant CONSORT extensions. See www.consort-spirit.org.

2. STATISTICAL ANALYSIS PLAN

STUDY TITLE:

A randomized, double-blind, placebo-controlled study to assess the efficacy of CaroRite™ on psychological well-being, oxidative stress, and quality of life in healthy individuals

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1. Abbreviations

Abbreviation	Full form
AE	Adverse event
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
CRF	Case report form
CRO	Contract research organization
CTCAE	Common Terminology Criteria for Adverse Events
E-CRF	Electronic CRF
GCP	Good Clinical Practice
ICH	International Council for Harmonization of Technical Requirements for Pharmaceutical for Human Use
ICH-GCP	International Conference on Harmonization-Good Clinical Practices
IP	Investigational product
ITT	Intention to treat subset
LOCF	Last observation carried forward
TG	Triglyceride
OC	Observed cases
PP	Per-Protocol
PR	Pulse Rate
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Source Document
PGWBI	Psychological General Well-Being Index
PSQI	Pittsburgh Sleep Quality Index
SF-36	36 Item Short Form Health Survey
WC	Waist circumference

2. Objectives

2.1 Primary Objective

Assess the impact of the Investigational Product (IP) on psychological well-being using the Psychological General Well-Being Index (PGWBI).

(Timeframe: Day 0, Day 30, Day 60, Day 90)

2.2 Secondary Objectives(s)

1. To assess the impact of the IP on: (Timeframe – Day 0 and Day 90)
    - Antioxidant capacity of plasma cells as measured with the 8-Isoprostanes
    - Immunomodulation using Salivary Immunoglobulin A (IgA)

2. To assess the impact of the IP on: (Timeframe – Day 0, Day 30, Day 60, Day 90)

    - Quality of life using 36 Item Short Form Health Survey (SF-36)
    - Sleep quality using Pittsburgh Sleep Quality Index (PSQI)

3. Endpoints

3.1 Primary Endpoint

- Psychological health as assessed by the increase in the Psychological General Well-being Index (PGWBI) scores at day 90

3.2 Secondary Endpoints

- Oxidative stress as assessed by decrease in serum levels of 8-Isoprostanes at day 90
- Immune Status as assessed by the increase in salivary IgA levels at day 90
- Quality of life as assessed by increase in the scores of SF-36 questionnaires at Day 90.
- Sleep quality as assessed by the decrease in the Pittsburgh Sleep Quality Index (PSQI) score at Day 90.

3.3 Safety Endpoints

Safety evaluation will include assessment of the following parameters:

- No significant change in the vitals (BP and PR) after IP consumption compared to the placebo. (Time frame: Day 0, Day 30, Day 60, Day 90)
- No significant difference in the number of adverse events/serious adverse events occurred during the study after IP consumption compared to placebo. (Time frame: throughout the study)

4. Analysis Sets

- **Intention to Treat (ITT) subset**

  The safety analysis will be done on the ITT population defined as all the enrolled participants who will take at least one dose of the study product.

- **Modified Intention to Treat (mITT) subset**

  The efficacy analysis will be done on all mITT research participants who have completed at least one post-baseline efficacy assessment.

- **Per protocol subset (PP)**

  All research participants without any major protocol violation will be included in the PP population and whose CRF is complete as requested. This population subset will also be used for efficacy analyses for robustness check.

5. Data Handling

Data entry into e-CRF must be performed using primary source data listed in source identification log and as per instructions given at the time of e-CRF training that will be documented by the Clinical Data Manager. Data entry will be made as per source document (SD) without interpretation or modification to maintain consistency and accuracy of the data. Study-specific data entry in e-CRF will be performed by the assigned study coordinator. Apart from visit specific data, which would primarily be

captured on paper SD would also be captured in e-CRF directly from the original source or as per the specific study requirement.

Investigators will maintain the Site Master File, Source Documents, and the Participant Files according to the ICH-GCP guidelines at the site.

6. Procedure for accounting missing and spurious data

The last observation carried forward (LOCF) will be the primary method for missing data imputation. The LOCF will be performed only in those cases where at least one post-baseline assessment data is available. The spurious data will be treated as missing data, and the same method will be applied for imputation for mITT Population.

For primary analysis with PP population no imputation will be performed, and analyses will be conducted on the observed cases (OC) only.

7. Statistical methods

Each treatment group will undergo comparison with the placebo across all time points. Additionally, within each group, the treatment arms will be assessed for changes from baseline to specific post baseline time points. Furthermore, comparisons between groups will evaluate changes from baseline to these specific time points within each group in contrast to the placebo arms.

Primary and secondary efficacy outcomes will be analyzed using the mITT & PP population, with PP population supportive to the mITT analyses. Safety variables will be assessed as per ITT population.

7.1 Normality Assessment

Endpoint data will be visually (graphically) assessed for skewness and kurtosis, and it will be determined if data are reasonably approximate to normal distribution or not. In case of sharp deviation from normality through visual assessment, distribution of the data will be further checked and presented for normality using Shapiro Wilk/Kolmogorov–Smirnov

test. In case data would be having sharp deviation from normality, non-parametric tests will be used as a supportive analysis.

7.2 Sample Size Calculation

Considering that study's primary outcome PGWBI is psychological in nature, a moderate effect size is expected. Hence for the Cohen's D value of 0.4 with an alpha error 0.05 and 80% power, the sample size of 28/group having degree of freedom=27, has been estimated. With the optimisation of the sample size, the study plans to enrol participants with at least not more than thirty-two participants/ group to have minimum of thirty participants each in CaroRite as well as placebo group.

7.3 Descriptive Methods

All continuous variables will be summarized by presenting the number of subjects, mean and its 95% CI, standard deviation, median, minimum, and maximum. Categorical variables will be presented as frequencies and percentages. Summary tables with p-value for each timepoint will be presented by treatment and visit, when applicable.
Change from baseline will be calculated as the amount of change between baseline and Day 30, Day 60, and Day 90.

This derived variable is calculated using the following formula, where x is time and T_0 is baseline:

$$\text{Change from baseline} = (\text{Result at } T_x - \text{Result at } T_0)$$

7.4 Statistical Tests

Unless otherwise specified, all statistical tests will be two-sided and will be performed with a significant level of 0.05. Confidence intervals (CIs) will be two-sided with 95% coverage.

7.3 Participant Disposition

Number of subjects screened, screening Failure, enrolled, randomized, and followed up at different visits will be presented along with details of discontinued/withdrawn subjects. Also, participants considered for efficacy and safety data analysis will be represented.

7.5 Demographic and Baseline Information

Continuous variables (including age, height, weight, waist circumference, and BMI) will be summarized using summary statistics (number of observations, mean and its 95% C.I; standard deviation, median, minimum, and maximum) by treatment group. Categorical variables like gender will be summarized using frequencies and percentages by treatment group.

The student's t-test for continuous and Chi-square/Fisher's exact test for categorical variables will be used to compare the baseline demographic data between groups.

8. Statistical Analysis of Endpoints

8.1 Primary Efficacy Endpoints

Change from the baseline in the individual domain score namely anxiety, depression, positive well-being, self-control, general health, vitality and the global PGWBI score to day 30, 60 and 90 will be compared between the IP and placebo groups using ANCOVA. The dependent variable will be the change value; and the treatment will be the factor of interest, and the baseline value will be used as a covariate. 95% Cis, LS means, and p-values will be reported. Within group comparisons of the baseline with Day 30, 60 and 90 visits' will be compared by a paired t-test. The student's t-test for will be used to compare unpaired data for comparing summary statistics at different visits.

Primary Analysis will be performed on mITT & PP Population.

8.2 Secondary Efficacy Endpoints

Secondary efficacy variables; the serum levels as well as the change in Salivary IgA Levels from baseline to day 90 will be will be compared between the IP and placebo groups using ANCOVA. The SF-36-dimension score and PSQI seven components and global Score from baseline to day 30, 60 and 90 days along with the mean change from baseline in serum levels, Salivary IgA Levels, SF-36-dimension score, and PSQI seven components and Global Score at day 30, 60 and 90 will be compared between the IP and placebo groups using ANCOVA. The dependent variable will be the change value; and the treatment will be the factor of interest, and the baseline value will be used as a covariate. 95% C.I; LS means, and p-values will be reported. Within group comparisons of the baseline with Day 30, 60 and 90 visits' will be compared by a paired t-test. The student's t-test for will be used to compare unpaired data for comparing summary statistics at different visits.

Secondary Analysis will be performed on mITT & PP Population.

8.3 Safety Analysis

Summary statistics for actual as well as mean change from baseline for vital signs (Pulse rate and blood pressure) will be summarized descriptively at all scheduled visits (Day 0, Day30, Day 60, and Day 90).

AEs/SAEs will be monitored and documented throughout the study duration. At the end of study, analysis will be conducted, and summaries of AEs/SAEs will be provided. AEs will also be summarised by each severity and by relationship grade.

Safety Analysis will be performed on ITT Population.

8.4 Interim Analysis

An interim analysis will be slated for thirty subjects for the following parameters.

1) psychological well-being using the Psychological General Well-Being Index (PGWBI).
2) Quality of life using SF-36
3) Sleep quality using Pittsburgh Sleep Quality Index (PSQI)

(Timeframe: Day 0, Day 30, Day 60, Day 90)

9. Listing of Tables

- Participant Disposition
- Demographical and baseline characteristics with comparison of demographic and baseline characteristics (with p-value) for ITT population
- Profile of Medical/Medication History if any
- Descriptive summary for IP Accountability and Compliance
- Descriptive summary for primary, secondary & safety variables (with p-value)
- Change in Domain and total PGWBI Score on Day 30, Day 60, and Day 90 from baseline (Day 0)
- Change in serum Levels, on Day 30, Day 60, and Day 90 from baseline (Day 0)
- Change in Salivary IgA Levels score on Day 30, Day 60, and Day 90 from baseline (Day 0)
- Change in SF-36-dimension score, on Day 30, Day 60, and Day 90 from baseline (Day 0)
- Change in PSQI seven components and Global Score on Day 30, Day 60, and Day 90 from baseline (Day 0)
- Summary of blood pressure and pulse rate on Day 0, Day 30, Day 60, and Day 90
- Change in blood pressure and pulse rate on Day 30, Day 60, and Day 90 from baseline (Day 0)
- Profile of AE/SAE events in each group (including by grade and relationship)

10. Listing of Graphs

- Line graph for mean change in domain and total PGWBI Score on Day 30, Day 60, and Day 90 from baseline (Day 0) by treatment group.
- Line graph for mean change in Serum Levels on Day 30, Day 60, and Day 90 from baseline (Day 0) by treatment group
- Line graph for mean change in Salivary IgA Levels score on Day 30, Day 60, and Day 90 from baseline (Day 0) by treatment group
- Line graph for mean change in SF-36-dimension score on Day 30, Day 60, and Day 90 from baseline (Day 0) by treatment group
- Line graph for mean change in PSQI seven components and Global Score on Day 30, Day 60, and Day 90 from baseline (Day 0) by treatment group

11. Software

- R/R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/./
- XLSTAT statistical and data analysis solution. New York, USA. https://www.xlstat.com./

3. STATISTICAL ANALYSIS PLAN (POST HOC ANALYSIS)

STUDY TITLE: A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy of Carorite™ on Psychological Well-Being, Oxidative Stress, And Quality of Life In Healthy Individuals

SAP Version: 1.0

SAP Date: 6th August 2025

Study Population:

Per Protocol (PP) population, N = 62.

Analysis Sets & Subgroups:

- Primary Analysis: All PP subjects.
- Subgroup Analyses:
  - Responder Analysis (PGWBI Total Score ≥ 1): Male subjects.
  - Age: <35 years vs. ≥35 years.
  - Gender: Male vs. Female.

Endpoints:

- Primary Endpoints:

  Change from Baseline to EOS (End of Study) in PGWBI Domains (Anxiety, Depressed Mood, Positive Wellbeing, Self Control, General Health, Vitality, Total Score)

  Change from Baseline to EOS in IgA Biomarkers (8-Isoprostanes, Salivary IgA)

- Secondary Endpoints:

  Correlation between change in PGWBI_Total Score and IgA biomarkers.

Statistical Methods:

Descriptive Statistics:

All continuous variables were summarized by presenting the number of subjects, mean and its 95% CI, standard deviation, median, minimum, and maximum. Categorical variables were presented as frequencies and percentages. Summary tables with p-value for each timepoint were presented by treatment and visit, when applicable.

Change from the baseline in each endpoint to day 90 was compared between the IP and placebo groups using ANCOVA. The dependent variable was the change value; and the treatment was the factor of interest, and the baseline value was used as a covariate.

This derived variable is calculated using the following formula, where x is time and T_0 is baseline:

Change from baseline = (Result at T_x - Result at T_0)

Within-Group Comparisons:

Within group comparisons were done by Paired t-test or Wilcoxon signed-rank test (if non-normal) for Baseline vs. Day 90 within each group/subgroup.

Between-Group Comparisons:

Independent t-test or Mann-Whitney U test (as appropriate) was applied for differences in change scores between CaroRite™ and Placebo arms, overall and within subgroups.

p-value < 0.05 was considered statistically significant.

Effect Size Calculation:

Effect Size was calculated using Cohen's d or equivalent for difference in change between groups.

Correlation Analysis:

Pearson/Spearman correlation coefficients for change in PGWBI Total Score vs. change in IgA biomarkers; significance evaluated at $p<0.05$.

Interpretation & Reporting:

Baseline comparability was documented for each variable (statistical difference at baseline).

Within-group and between-group significance was reported for each endpoint and subgroup.

Numerical change and effect size interpretation: positive/negative directionality was contextualized.

All results were tabulated as Mean (SD), P-value, and effect size.

Significant correlations between psychological and immunological endpoints were highlighted.

Missing data was described, but only complete cases were analyzed (PP).

All statistical analyses were performed using statistical software (e.g., R and XLSTAT), with two-sided hypothesis testing.

4. Table S1- Summary of Adverse Event

Adverse Event	CAR (n)	Placebo (n)	Total (n)
Body pain	3	2	5
Headache	1	1	2
Loose motion	1	0	1
Cold and cough	1	0	1
Dizziness due to blood sample collection	0	1	1
Total	**6**	**4**	**10**