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Oral Administration of Nicotinamide Mononucleotide with Bioenhancer BioPerine® Increases the Serum Concentration of Nicotinamide Adenine Dinucleotide in Healthy Human Volunteers: A Pilot, Open-Label, Cross-Over Study

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Abstract

Background: Bioenhancers augment the bioavailability of co-administered molecules without showing any significant effect on their own. Piperine, an alkaloid from *Piper nigrum*, is an established natural bioenhancer. Nicotinamide mononucleotide (NMN), an antiaging supplement, is the precursor of coenzyme nicotinamide adenine dinucleotide (NAD) that plays an important role in intracellular redox reactions. **Objective:** The study compared the serum concentrations of NAD in normal healthy participants, supplemented with NMN 500 mg and NMN 500 mg + 5 mg BioPerine® (95% piperine). **Methods:** In a randomized, open-label, crossover study, NMN (500 mg) was compared to NMN + BioPerine® (500 mg + 5 mg) in 6 healthy adults, aged 18 - 45 years. The participants received a single oral dose of NMN or NMN + BioPerine® capsules with 240 mL water, and blood samples were collected over 8hr. After a 4-day washout period, the same procedures were repeated as per the crossover design. Total NAD (NAD_{total}), including oxidized NAD (the oxidized) and its reduced form NADH, was measured in human serum samples. **Results:** The maximum concentration (C_{max}) of NAD in serum was higher with NMN + BioPerine® (282 pmol/mL) compared to NMN (246 pmol/mL)

alone. In the presence of BioPerine®, the NAD concentrations reached 257 pmol/mL during the first 2 hr, whereas a comparable serum concentration (246 pmol/mL) was attained only after 6 hr in NMN alone. The $AUC_{0\text{-}8hr}$ was 1738 pmol/mL/hr in NMN compared to 2004 pmol/mL/hr in NMN+ BioPerine®. The time to reach peak concentration ($t_{1/2}$) was similar (6hr) in both groups. No clinically relevant adverse events (AE) were observed, and safety parameters remained within normal ranges in all the participants with both formulations. **Conclusion:** These results reveal that BioPerine® can effectively increase the NAD concentrations in the serum following NMN supplementation in healthy volunteers. The present study was registered prospectively with the Clinical Trials Registry-India (CTRI/2023/11/059982).

Keywords

Bioavailability, BioPerine®, Nicotinamide Mononucleotide, Nicotinamide Adenine Dinucleotide, Bioenhancer

1. Introduction

Black pepper, scientifically known as *Piper nigrum* L., is a commonly used spice renowned for its pungent component, piperine. Beyond its culinary application, pepper possesses significant medicinal and preservative activities [1] [2]. Several studies have revealed that piperine facilitates the absorption and bioavailability of herbal extracts, phytochemicals, vitamins, and other molecules [3] [4].

A bioenhancer is a substance that can increase the absorption of a co-administered compound, without exerting its inherent pharmacological effects [5]. The concept of bioenhancers originated in the traditional Ayurvedic system of medicine, where black pepper, long pepper, and ginger were used in several formulations. Bioenhancers may also improve efficacy, reduce toxicity, minimize the incidence of drug resistance and lower treatment cost [6] [7].

Piperine has been extensively studied and validated as a bioavailability enhancer [7] [8]. Piperine inhibits cytochrome P450 enzymes and UDP-glucuronyltransferase, which are responsible for the metabolism of most compounds [9] [10]. It increases the absorption of co-administered molecules by vasodilation of gut tissues and inhibits the P-glycoprotein efflux pump, thus increasing their cellular concentration [11]. By stimulating gamma-glutamyl transpeptidase, piperine enhances the uptake of amino acids [12]. However, it was also noted that piperine was not a universal bioenhancer and hence, it is imperative to study its effect with various nutrients [13].

NAD plays a critical role in cellular metabolism, energy production, and DNA repair [14]. Circadian oscillation of NAD is observed in cells due to circadian regulation of nicotinamide phosphoribosyltransferase [15] [16]. Age-related health complications such as cardiovascular disease, diabetes, cancer, and Alzheimer's disease are directly related to decreased NAD levels in cells and tissues due to

dysfunction of systemic NAD^+ biosynthesis with age [17]-[19]. NAD levels decline with age due to an increase in NAD-consuming enzymes, and a reduction in NAD-synthesizing enzymes or both [20]. The steady loss of cellular functioning that occurs with aging reduces fitness and raises the risk of illness and death [21]. There is a surge in the use of supplements for graceful aging and a growing consumer demand for anti-aging health products [22]. NMN has garnered substantial interest in recent years, as a precursor for NAD [23]. Owing to its potential efficacy, NMN is now a common ingredient in many anti-aging health products [24]. By the end of 2027, the global NMN market is expected to grow from its 2020 valuation of US$253 million to US$386 million [25].

A common dosage for NMN supplements is around 250 - 500 mg/day; the dosage can vary depending on individual needs. In a clinical study, the absorption plateaus of NMN did not show any significant increase when 300 mg, 600 mg, and 900 mg were used in healthy individuals, suggesting that the absorption of NMN is not always directly proportional to its dosage [26]. Recently, co-administration of NMN with resveratrol, or ginsenoside was found to increase the NAD levels in tissues [27]. Furthermore, orally administered NMN is extensively metabolized in the stomach and liver, resulting in excretion, as nicotinamide. Thus, it is imperative to create an NMN formulation that can increase its bioavailability [28]. This study is the first among the project "BioAlphabet", initiated by Sami-Sabinsa Group to understand the effect of BioPerine® on different supplement absorption. We explored the NAD concentrations in serum following oral administration of NMN+ BioPerine® (95% piperine) compared to NMN alone.

2. Materials and Methods

2.1. Ethics

The study was approved by the ACE Independent Ethics Committee, Bangalore on 07-Nov-2023 under protocol number: PRPL-NMN-02-2023. Each participant signed an informed consent form to take part in the research. The study was performed according to the Indian Council of Medical Research (ICMR) ethical guidelines for biomedical research on human participants, the International Council for Harmonization (ICH) Guidelines for Good Clinical Practices (E6-R2) 2016, Central Drugs Standard Control Organization (CDSCO's) New Drugs and Clinical Trials Rules 2019 G.S.R. 227 (E), Good Clinical Practices for Clinical Research in India, CDSCO Guidelines for BA/BE Studies-March 2005, and the principles enunciated as per the Declaration of Helsinki and in compliance with Good Laboratory Practice (GLP).

2.2. Participants

Inclusion criteria: The study included healthy adult volunteers in the age group of 18 - 45 years. The participants had no clinically significant medical history, and their physical examination results and laboratory profiles, were normal as deemed by the investigator. The participants were included only if they agreed not to

consume coffee, tea, cola, chocolate, or any other xanthine-containing food or drinks, three days before the start of the study and during the washout period. Only non-pregnant, non-lactating females and postmenopausal women were included in the study.

Exclusion criteria: Individuals with a history of uncontrolled systemic diseases, chronic alcoholism/smoking/drug abuse, hypersensitivity to NMN and BioPerine® or their excipients were excluded from the study. Other criteria for exclusion from the study were recent intake of vitamins/mineral supplements/herbal medicines/prescription drugs/OTC medicines/tobacco within 14 days potentially affecting NMN pharmacokinetics, recent blood donation or significant blood loss within the last month, positive report for hepatitis B, hepatitis C, or HIV 1 & 2. Participants were excluded if they tested positive for the Drugs of Abuse Test during Screening or Period I or Period II.

2.3. Sample Size

A total of 6 volunteers were included in the study. Eligible volunteers were randomized in a 1:1 ratio to NMN 500 mg or NMN 500 mg + 5 mg BioPerine® groups.

2.4. Study Design

The study was conducted as a pilot, two-arm, two-period, cross-over, open-label, single-center, comparative trial from 21-Nov-2023 to 29-Nov-2023, to investigate the effect of BioPerine® on the blood NAD concentrations following oral supplementation of NMN in normal healthy volunteers. The primary objective of the study was to compare the NAD levels in the blood, following NMN 500 mg and NMN 500 mg + 5 mg BioPerine® supplementation. The secondary objective was to monitor the safety and tolerability of the investigational products by the incidence of any AE.

The study consisted of two periods (24 hr each) with a washout period of 4 days in between (**Figure 1**). All the eligible volunteers were randomized in a 1:1 ratio to receive NMN 500 mg or NMN 500 mg + 5 mg BioPerine®. In each study period, the participants were accommodated in the clinical facility on the day of dosing. The study participants were fasted overnight for at least 8 hr before administration. A single oral dose of NMN 500 mg or NMN 500 mg + 5 mg BioPerine® was administered to the participants in a sitting posture at a fixed time with 240 ± 05 mL of water. Blood samples were collected over eight-hour time points: pre-dose (0 hr), 0.25 hr, 0.50 hr, 0.75 hr, 1.00 hr, 1.50 hr, 2.00 hr, 3.00 hr, 4.00 hr, 6.00 hr, and 8.00 hr, post-dose. Blood samples were obtained through an indwelling cannula placed in a forearm vein. All serum samples were collected using a pre-labeled gel tube at the scheduled time with a window period of 2 minutes. Before each sample collection from the cannula, about 0.5 mL of blood was drawn and discarded. The samples collected at the clinical site were allowed to stand for 30 minutes and centrifuged at 3000 rpm for 20 minutes to separate the serum. The serum was aliquoted into two portions and stored at −20˚C at

the clinical site until it was shipped and transferred to the bioanalytical lab for further analysis.

NAD_{total} (NAD^+ and NADH) in human serum samples was measured using the NAD/NADH quantification kit (Catalogue Number, MAK037, Sigma Aldrich, USA). Laboratory safety assessments (CBC, RBS, HbA1C, LFT, KFT, Thyroid Function Test, Lipid profile & Serology, Urine routine with electrolytes) were carried out at screening and the end of the study. Vital parameters (body temperature, pulse rate, respiratory rate, and blood pressure) were done throughout the study. Participants received a standardized meal at 04.00- and 08.00-hr post-dose with a time flexibility of +15 min from the schedule. Drinking water was prohibited from 1hr before dosing until 1hr after dosing, and at all other times, drinking water was permitted as required. All the participants were instructed to avoid coffee, tea, cola, chocolate, or any other xanthine-containing food or drinks during their stay in the clinic and not to consume citrus fruits (such as lime, lemon, and orange) containing food items, alcohol and tobacco starting from 48hr before dosing and during the entire duration of the study. Grapefruit or its products were prohibited from 10 days before the start and during the study.

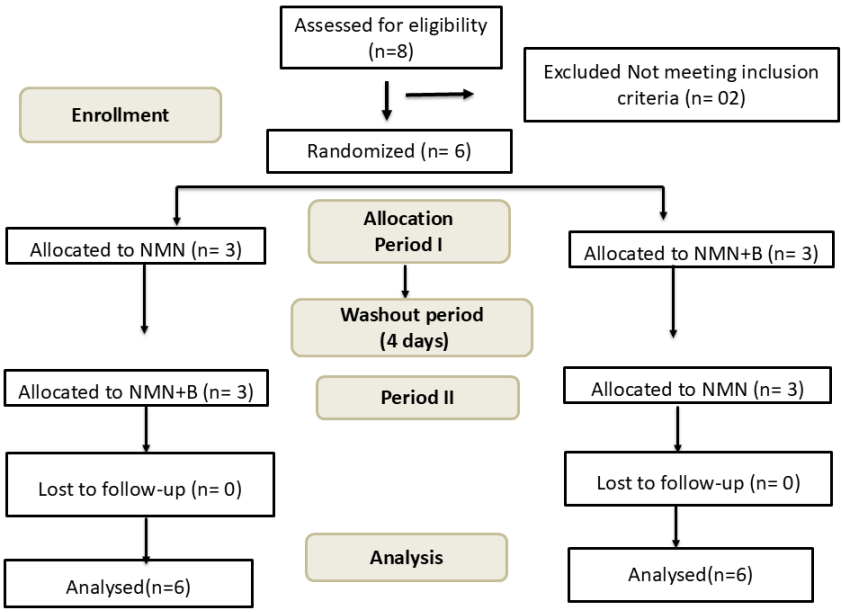

Figure 1. Flow diagram of the study as per the consolidated standards of reporting trials guidelines (NMN+ B: Nicotinamide Mononucleotide 500 mg + 5 mg BioPerine®).

2.5. NAD/NADH Quantification

NAD is an electron acceptor and interconverts to its reduced form NADH (hydride), which is a fundamental process in metabolism. NAD_{total} in human serum samples, which included NAD and its reduced form NADH, was measured using the NAD/NADH quantification kit as per the manufacturer's instruction (Catalogue Number MAK037, Sigma Aldrich, USA).

2.6. Chemicals/Reagents Preparation

Reagent vials were briefly centrifuged before opening and ultrapure water was used to prepare the reagents. NAD cycling buffer was allowed to reach room temperature before use and the NAD cycling enzyme mix was prepared by reconstituting in 220 µL of NAD cycling buffer. Adequate NAD cycling enzyme mix (2 µL per assay) was aliquoted as per the requirement and the stock solution was immediately frozen at −70˚C for future use. The enzymes were stable for up to 2 months when stored at −70˚C after reconstitution. NADH developer was reconstituted in 1.2 mL of water and mixed well by pipetting without vortexing. NADH standard was reconstituted in 200 µL of DMSO to generate 1mM solution and mixed well by pipetting and stored at −20˚C.

2.7. Bioanalytical Procedures

In a 96-well assay plate, 50 µL of serum was added, followed by 100 µL of master reaction mixture (containing 98 µL of NAD cycling buffer and 2 µL of NAD cycling enzyme) and mixed well with a pipette and incubated for 5min at room temperature to convert NAD to NADH. Then 10 µL of NADH developer was added to each well and incubated at room temperature for 4 hr, and the absorbance was measured at 450 nm with a microplate reader. The total amount of NAD present in serum samples (pmol) was determined from the NADH standard curve.

The NADH standards were prepared by diluting 10µL of the 1mM NADH standard with 990 µL of the NADH/NAD extraction buffer to generate a 10µM standard solution. Subsequently, 0, 2, 4, 6, 8, and 10 µL of the 10µM NADH standard were added to a 96-well plate, resulting in 0 (blank), 20, 40, 60, 80, and 100 pmol/well standards. The volume in each well was made up to 50 µL with NADH/NAD extraction buffer. The standard graphs were repeated thrice (Figure 2). An average of 10% of samples were randomly repeated to ensure the experimental variability.

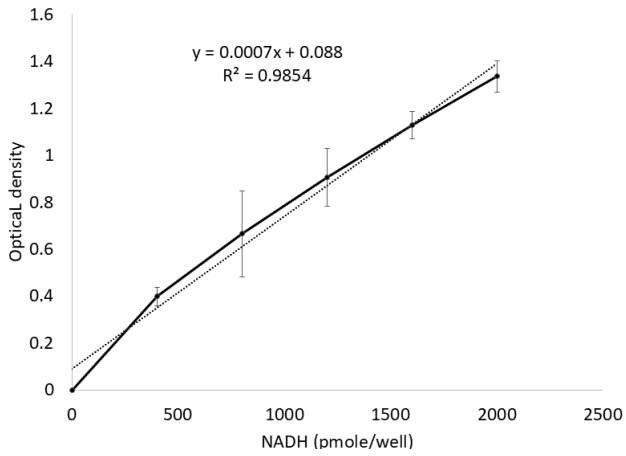

Figure 2. The standard curve was repeated thrice, and the average values and Standard deviation are plotted in the graph. The unknown concentrations were calculated from the standard curve.

2.8. Safety

Laboratory safety assessment (CBC, RBS, HbA1C, LFT, KFT, Thyroid Function Test, Lipid profile & Serology, Urine routine with electrolytes) was assessed at screening, and end of the study. Vital parameters (body temperature, pulse rate, respiratory rate, and blood pressure) were assessed at all the scheduled visits. Well-being and AE were monitored throughout the study.

2.9. Pharmacokinetics (PK)

PK analysis was conducted using the freely available PK calculator web tool https://dash.gallery/dash-pk-calc/. The differences within the groups were assessed using a t-test or Wilcoxon signed-rank test based on the normal distribution of data. A separate analysis was performed for each of the two study groups. The concentration of NMN was used as the basis to assess the following PK parameters: Half-life ($t_{1/2}$), Area Under the Curve ($AUC_{0\text{-}8hr}$, $AUC_{0\text{-}inf}$) % extrapolation, Maximum concentration reached (C_{max}), and Time to reach the maximum concentration (T_{max}). The concentrations of NMN in the groups that consumed NMN 500mg and NMN 500mg+ 5mg BioPerine® formulation were used as the primary parameter to compare the difference in AUC and study outcomes.

2.10. Statistical Evaluation

The data analysis was performed as per the Statistical Analysis Plan (SAP) with a 5% significance level. The final data was analyzed using SAS® version 9.4 and Microsoft Excel software 2021 version.

3. Results

A total of six healthy male volunteers were enrolled in the study. All the participants completed the study and were included in the data analysis. The age of the participants ranged from 18 to 45 years. All of them had weights in the range of 55 - 69 kg and a BMI of 21.5 to 24.7 kg/m^2, which was in the normal range. The CONSORT flow diagram is represented in Figure 1, and the demographic data in Table 1.

Table 1. Demographic details.

Parameters	(Mean ± SD)	(Min, Max)
Age (Years)	33.67 ± 10.25	19, 45
Height (cm)	163 ± 6.13	155, 170
Weight (kg)	61.8 ± 6.34	55, 69
BMI (kg/m^2)	23.22 ± 1.16	21.5, 24.7
Pulse Rate (Beats/min)	71.67 ± 1.37	70, 74
Respiratory Rate (Breaths/min)	88.17 ± 12.28	68, 98
Systolic BP (mmHg)	128.33 ± 6.18	120, 139
Diastolic BP (mmHg)	81.33 ± 3.59	74, 94
Body Temperature (˚F)	96.67 ± 0.47	96, 97

3.1. Bioanalytical Results

The amount of NAD_{total} (pmol/mL) present in the serum samples was determined from the standard curve (Figure 2).

BioPerine® significantly improved the pharmacokinetic parameters of NMN

NAD_{total} in human serum samples, which included NAD and its reduced form NADH, was measured using the NAD/NADH quantification kit as described in the text. At 0 hr, the average concentration of NAD_{total} was 136.8 pmol/mL in NMN and 122.6 pmol/mL in NMN+ BioPerine®. The C_{max} was 246 pmol/mL with NMN and 282 pmol/mL with NMN+ BioPerine® groups. The percentage increase in serum concentration of NAD from 0 hr to C_{max} was 79.82% for NMN and 130.02% for NMN+ BioPerine®. The change in total NAD concentration from dosing to C_{max} was 159.4 pmol/mL in NMN+ BioPerine® and 109.2 pmol/mL in NMN (45.97% increase in the presence of BioPerine®) (Figure 3).

The concentration of the metabolites increased in both groups with time. After two hours, the average concentration of NAD_{total} in the NMN+ BioPerine® group increased to 257 pmol/mL while it was 195.2 pmol/mL in the NMN group.

The time to reach peak concentration (t_{max}) was 6 hr in both groups (Table 2). The addition of BioPerine® to NMN enhanced the NAD_{total} in serum by 14.6%, which could directly influence the pharmacological responses of NMN.

The area under the curve ($AUC_{0\text{-}8hr}$) was also considerably higher in the NMN+ BioPerine® group at 2004 pmol/mL/hr compared to 1738 pmol/mL/hr in the NMN group, while the $AUC_{0\text{-}inf}$ was 3558 pmol/mL/hr and 3396 pmol/mL/hr with and without BioPerine® respectively. The PK parameters were found to be significantly better in NMN+ BioPerine® compared to NMN (overall p-value of 0.000594 based on t-test analysis). The time required for NAD level to reduce to half of its initial value was 7 hr for NMN and 6.1 hr for NMN+ BioPerine® (Table 2).

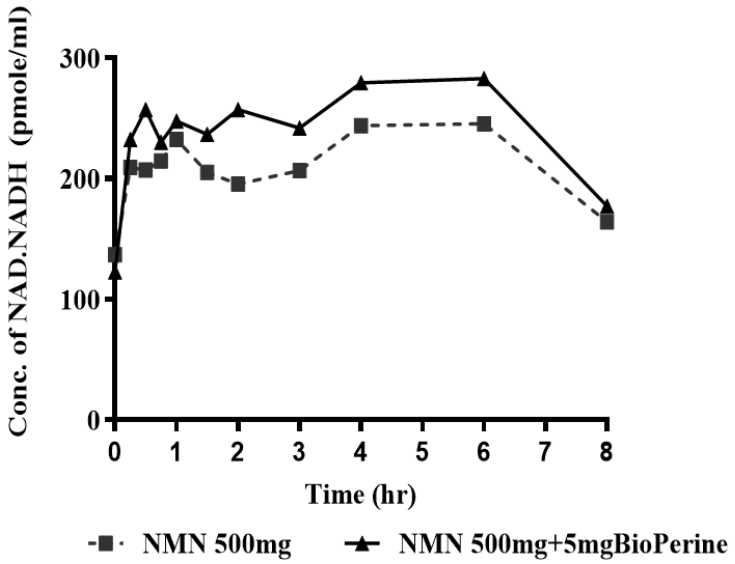

Figure 3. Average concentrations of NAD. NADH in NMN 500 mg and NMN 500 mg + BioPerine® 5 mg groups at different time points.

Table 2. Pharmacokinetic parameters of NMN.

Parameter	NMN 500 mg	NMN 500 mg + 5 mg BioPerine®	% Change
C_{max} (pmol/mL)	246	282	14.6
$AUC_{0\text{-}8hr}$ (pmol/mL/hr)	1738	2004	15.3
$AUC_{0\text{-}inf}$ (pmol/mL/hr)	3396	3558	4.8
$T_{1/2}$ (hr)	7	6.1	−12.85
T_{max} (hr)	6	6	0

3.2. Safety

NMN (500 mg) and BioPerine® (5 mg) were found to be safe and well tolerated in healthy adult participants under fasting conditions. There was no significant variability in the safety parameters during the treatment period. No severe, serious, or life-threatening AE were reported or observed during the study (Table 3).

Table 3. Laboratory safety parameters.

Parameter	Screening (Mean ± SD)	EOS (Mean ± SD)
Lipid Profile		
Total Cholesterol (mg/dL)	177.33 ± 26.51	183.5 ± 29.75
Triglycerides (mg/dL)	119.16 ± 74.25	178.83 ± 135.71
HDL-C (mg/dL)	46.5 ± 11.51	44.5 ± 12.18
LDL-C (mg/dL)	106.5 ± 20.02	102.83 ± 10.99
VLDL-C (mg/dL)	25.16 ± 15.17	35.5 ± 27.01
Complete Blood Count		
Hemoglobin (gram/dL)	14.96 ± 0.65	15.06 ± 0.70
RBC (million/μL)	4.77 ± 0.38	4.85 ± 0.35
Packed Cell Volume (%)	43.33 ± 1.69	44.03 ± 2.45
Mean Cell Volume (fL)	91.16 ± 8.07	90.72 ± 7.94
MCH (pg)	31 ± 2.58	30.51 ± 2.36
MCHC (%)	34 ± 0	33.43 ± 0.63
Total Leukocyte Count (cell/cm)	6786.667 ± 1241.017	6108.33 ± 1172.78
Neutrophils (%)	59.45 ± 4.83	52.51 ± 6.33
Lymphocytes (%)	29.75 ± 3.63	34.3 ± 5.34
Monocytes (%)	7.13 ± 1.34	8.35 ± 1.13
Eosinophils (%)	3.33 ± 2.51	4.31 ± 2.53
Basophils (%)	0.33 ± 0.11	0.36 ± 0.13
Platelet Count (lakhs/cumm)	2.63 ± 0.39	2.72 ± 0.45
Mean Platelet Volume (fL)	8.63 ± 1.01	8.83 ± 0.96
Urine Routine		
Color	Pale Yellow	Pale Yellow
Transparency	Clear	Clear
PH	6.25 ± 0.25	6.16 ± 0.23
Specific Gravity	1.01 ± 0.006	1.02 ± 0.001
Glucose	Absent	Absent
Protein	Absent	Absent
Ketone Bodies	Absent	Absent
Urobilinogen	Normal	Normal
Nitrites	Absent	Absent

Continued

Pus cells/HPF	1 - 5	1 - 4
Epithelial cells	1 - 5	1 - 8
RBC	2 - 3	1 - 3
Crystals	Absent	Absent
Cast	Absent	Absent
Bacteria	Absent	Absent
Thyroid Function		
T3 (ng/dL)	1.47 ± 0.19	1.27 ± 0.14
T4 (ng/dL)	8.89 ± 3.60	9.22 ± 1.10
TSH (ng/dL)	2.89 ± 1.48	2.44 ± 1.66
HbA1C	5.93 ± 0.40	6.1 ± 0.52
Renal Function		
Urea (mg/dL)	24.83 ± 6.28	21.66 ± 4.02
BUN (mg/dL)	11.51 ± 2.82	9.83 ± 1.95
Uric Acid (mg/dL)	7.85 ± 0.80	6.15 ± 0.83
Serum Creatinine (mg/dL)	0.85 ± 0.13	0.84 ± 0.10
Electrolytes		
Sodium (mmol/L)	137 ± 0.81	138.5 ± 0.76
Potassium (mmol/L)	4.33 ± 0.32	3.96 ± 0.13
Chloride (mmol/L)	102.5 ± 0.76	102.16 ± 2.33
Urine Electrolytes		
Sodium (mmol/L)	191.5 ± 61.29	163 ± 26.59
Potassium (mmol/L)	30.83 ± 10.99	10.41 ± 2.19
Chloride (mmol/L)	192.16 ± 52.07	152.16 ± 19.51
FBS (mg/dL)	91.33 ± 9.44	100.83 ± 15.91
Liver Function Test		
Total Bilirubin (mg/dL)	0.66 ± 0.19	0.48 ± 0.14
SGOT (U/L)	23.83 ± 2.96	15.5 ± 1.11
SGPT (U/L)	20.83 ± 5.30	20 ± 4.83
GGT (U/L)	38.5 ± 8.99	33.5 ± 8.53
Alkaline Phosphatase (IU/L)	145.16 ± 183.42	52.5 ± 7.45
Albumin (g/dL)	4.71 ± 0.43	4.15 ± 0.15

The values related to the laboratory safety parameters from screening to the end of the study (EOS) are represented as Mean ± SD. MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RBC: Red blood cell count, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Low-density lipoprotein cholesterol, HPF: High power field microscope, TSH: Thyroid-Stimulating Hormone, BUN: Blood Urea Nitrogen, FBS: Fasting Blood Sugar, SGOT: serum glutamic-oxaloacetic transaminase, SGPT: Serum Glutamic Pyruvic Transaminase, GGT: Gamma-glutamyl transferase.

4. Discussion

This randomized double-blind trial has shown that the serum concentration of NAD can be increased in the presence of a small quantity of piperine in the NMN supplement formulation. The supplement was safe, and no adverse reactions were observed in the study participants.

NAD is an electron acceptor and interconverts to its reduced form NADH (hydride), which is a fundamental process in metabolism. NMN, a precursor of NAD

is used as an antiaging supplement. NAD levels deplete in the body with age, and impact mitochondrial energy production, leading to oxidative stress, DNA damage, cognitive impairment, and inflammatory conditions [2]. In preclinical studies, NMN was reported to get absorbed in the intestine and immediately converted into NAD^+ in tissues. In the last few years, multiple clinical trials have shown an increase in NAD concentration in serum following oral supplementation of NMN [29]-[31]. The NAD_{total} and NADH in serum were analyzed using colorimetric method, mass spectrometry, or LC-MS/MS in these studies. In an efficacy and safety study of β-nicotinamide mononucleotide (NMN) supplementation in healthy adults, Yi et al. (2023) reported an increase in NAD_{total} concentrations in serum as analyzed by colorimetric method and safety up to a dose of 900 mg daily for 60 days [26].

In the present study, we used a validated colorimetric method for the analysis of NAD_{total} levels in serum, to understand the effect of piperine on NAD_{total} levels after NMN supplementation. BioPerine® is a patented extract derived from the fruits of black pepper (*Piper nigrum*), containing a minimum of 95% piperine. Piperine is known to improve nutrient absorption by stimulating the pancreatic digestive enzymes, thereby enhancing the digestive capacity, and notably decreasing the gastrointestinal food transit time which allows the nutrients and drugs to be absorbed more efficiently [32] [33]. It inhibits the metabolizing enzymes like cytochrome P450 and UDP-glucuronyltransferase which is responsible for the metabolism of nutrients [3].

BioPerine® is marketed by Sabinsa Corporation and has been used as a bioavailability enhancer for more than 20 years. BioPerine® has the remarkable ability to increase the bioavailability of co-administered nutrients. The bioenhancing activity of BioPerine® is mediated by modulating the enzymes responsible for metabolizing nutritional substances, by stimulating the activity of amino-acid transporters in the intestinal lining, inhibiting the p-glycoprotein, the "efflux pump" protein that prevents nutrients from entering the cells and decreases the intestinal production of glucuronic acid and by reducing the activity of glucuronyl transferase enzyme thereby permitting more of the substances to enter the body in their active form [11] [13] [34]. In the presence of BioPerine® (5 mg), the AUC of coenzyme Q10 was found to increase by 30% in human plasma compared to coenzyme Q10 alone [35]. Similar effects were also reported with 60% increase in serum beta-carotene levels in the presence of 5mg BioPerine® [36]. A recent detailed study on a rat model showed co-administration of piperine with curcumin resulted in a selective reduction in the expression of UDP-glucuronyltransferase (UGT) and sulfotransferases (SULT) a novel mechanism by which piperine enhances the bioavailability of curcumin. The result of this study suggests that UGTs and SULTs transform curcumin into biologically inactive metabolites. By inhibiting these enzymes, piperine ensures bioavailability of curcumin [37].

Piperine is a safe molecule at the concentration used for bio-enhancement. It is not genotoxic and does not induce any AE, even at 5 - 20 times higher doses than

the average human intake [38]. Piperine has been used along with vitamins, minerals, and nutrients as a bioavailability enhancer [39]-[41]. At the recommended dose of 5mg, oral BioPerine®, administration is not likely to have any safety concerns.

In the present study, we observed an increase in NAD in the serum of individuals consuming NMN+ BioPerine® compared to NMN. AUC serves as an indicator of drug availability in the body, representing the overall quantity of active drugs entering systemic circulation [42]. The $AUC_{0\text{-}8hr}$ of NAD_{total}, was 15.3% higher, while the C_{max} showed 14.6% improvement in the NMN+ BioPerine® group when compared to NMN, suggesting better availability of NAD in the presence of piperine.

Similar results of increased bioavailability of herbal extracts were observed with Curcumin [43], stilbenes [44], BioIron [45], and coenzyme Q10 levels [35] with BioPerine® in preclinical and clinical studies. The presence of piperine was shown to increase the oral bioavailability and immune response of Ginsenoside Rh2 by increasing the permeability and AUC and inhibiting the metabolism of Rh2 [46]. An *in vivo* study demonstrated the efficacy of piperine in enhancing the emodin's bioavailability by inhibiting glucuronidation which increased the AUC and C_{max} of emodin [47].

Our results concur with these studies and suggest that the addition of BioPerine® may be beneficial in increasing the NAD for cellular functions. NMN has been used at doses ranging from 250 to 900 mg per day, and its absorption did not show any significant increase from 300 mg to 900 mg used in healthy individuals [26]. We used the most common dose of 500 mg in our study, to get a detectable amount of NAD in the serum.

BioPerine® has been shown to be a safe bioenhancer for several nutrients [39]-[41]. This effect with NMN may help to reduce the required dosage of NMN, while maintaining the therapeutic efficacy [48]. Alternatively, it can help to deliver higher NAD in chronically deficient individuals.

As NAD levels reduce with age, the use of BioPerine® with NMN may help in maintaining the critical levels of NAD in geriatric individuals. Maintaining NAD levels provides numerous health benefits including cardiometabolic and immune health. Thus, the addition of BioPerine® to NMN could have wide ranging health benefits in individuals consuming the supplement. The bioenhancement observed in the present study was moderate, compared to the over 30% increase in bioavailability reported for other supplements [35] [43]. This could be because the study evaluated the bioenhancement effect after a single dose of NMN. Although it was not intentional all the volunteers recruited in the present study belonged to male gender. Gender related differences have been reported for NAD. Women are generally known to have lower NAD levels, and this can influence the PK and efficacy of NMN supplementation [49]-[51]. Future studies with longer dosage schedules of the NMN with BioPerine® in both male and female volunteers will help understand the long-term safety and enhancement effect of

BioPerine® on NMN.

5. Conclusion

The result of the present study suggests that BioPerine® can be used effectively to increase the NAD concentrations in the serum following NMN supplementation in healthy volunteers. BioPerine® has the potential to be used as a bio-enhancer when combined with NMN.

Authors' Contributions

AM, SM: Conceptualization, Funding, Reviewing.
TS: Principal Investigator.
SG, AKP, VN: Clinical trial monitoring, Reviewing.
LM: Protocol development, Analysis, Writing and Reviewing.

Conflicts of Interest/Competing Interests

AM, SM, TS, SG, AKP, VN, and LM are affiliated with Sami-Sabinsa Group Limited, which markets BioPerine®. TS is the principal investigator from TR Super Speciality Hospital, Bengaluru, Karnataka and has no conflict of interest.

Availability of Data and Material

All the data supporting the conclusions of this article are presented in the manuscript. Any additional requirement of the data can be requested from the corresponding author.

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