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ORIGINAL ARTICLE

Hydrogen-rich water reduces liver fat accumulation and improves liver enzyme profiles in patients with non-alcoholic fatty liver disease: a randomized controlled pilot trial

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KEYWORDS
Non-alcoholic fatty liver disease;
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Dual-echo MRI;
Fat accumulation

Summary
Background and aims: While non-alcoholic fatty liver disease (NAFLD) is rapidly becoming the most common liver disease worldwide, its treatment remains elusive. Since metabolic impairment plays a major role in NAFLD pathogenesis, any pharmaceuticals, such as molecular hydrogen (H_2), that advance lipid and glucose metabolism could be appropriate to tackle this complex condition. The aim of this study was to analyze the effects of 28-day hydrogen-rich water intake on liver fat deposition, body composition and lab chemistry profiles in overweight patients suffering from mild-to-moderate NAFLD.
Methods: Twelve overweight outpatients with NAFLD (age 56.2 ± 10.0 years; body mass index 37.7 ± 5.3 kg/m^2; 7 women and 5 men) voluntarily participated in this double-blind, placebo-controlled, crossover trial. All patients were allocated to receive either 1 L per day of hydrogen-rich water (HRW) or placebo water for 28 days. The study was registered at ClinicalTrials.gov (ID NCT03625362).

Abbreviations: γ-GT, gamma-glutamyltranspeptidase; AFIarm, fat index; ALP, alkalinephosphatase; ALT, alaninetransaminase; ASTaspartate, transaminase; CK, creatinekinase; H_2, molecularhydrogen; HDL, high-densitylipoprotein; HRW, hydrogen-richwater; MRI, magneticresonance imaging; LDL, low-densitylipoprotein; NAFLD, non-alcoholicfatty liver disease; ROI, regions-of-interest; S_{IP}, signalintensity on the in-phase; S_{OP}, signalintensity on the out-phase; TE, echotimes.

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Results: Dual-echo MRI revealed that HRW significantly reduced liver fat accumulation in individual liver regions-of-interest at 28-day follow-up, as compared to placebo administration ($P < 0.05$). Baseline liver fat content was reduced from 284.0 ± 118.1 mM to 256.5 ± 108.3 mM after hydrogen treatment at 28-day follow-up (percent change 2.9%; 95% CI from 0.5 to 5.5). Serum aspartate transaminase levels dropped by 10.0% (95% CI; from −23.2 to 3.4) after hydrogen treatment at 28-day follow-up. No significant differences were observed between treatment groups in either weight or body composition among participants.
Conclusions: Although preliminary, the results of this trial perhaps nominate HRW as an adjuvant treatment for mild-to-moderate NAFLD. These observations provide a rationale for further clinical trials to establish safety and efficacy of molecular hydrogen in NAFLD.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder characterized by liver fat deposition due to causes other than excessive alcohol consumption [1]. NAFLD can cause many liver dysfunction-related symptoms and signs, with the disease may progress to non-alcoholic steatohepatitis, a condition marked by liver inflammation, fibrosis, and irreversible damage. NAFLD is usually accompanied by insulin resistance and obesity, with up to 30% of the population in industrialized countries have NAFLD [2]. While NAFLD is rapidly becoming the most common liver disease worldwide, its treatment remains elusive and directed toward correction of the risk factors [3]. Since metabolic impairment plays a major role in NAFLD pathogenesis [4], any pharmaceuticals that advance lipid and glucose metabolism could be appropriate to tackle this complex condition. Molecular hydrogen (H_2) has recently emerged as a novel 'pro-metabolic' agent that might positively affect liver health. Supplemental hydrogen improves blood lipid profiles and insulin resistance in overweight women [5], patients with type 2 diabetes [6], and in subjects with potential metabolic syndrome [7]. In addition, drinking hydrogen-rich water (HRW) reduces hepatic oxidative stress and alleviated fatty liver damage in rodents [8,9]. However, no human studies so far evaluated its effectiveness to alter liver steatosis in patients with NAFLD. In this pilot trial, we analyzed the effects of 28-day HRW intake on liver fat deposition, body composition and lab chemistry profiles in overweight patients suffering from mild-to-moderate NAFLD.

Patients and methods

Patients

Twelve overweight outpatients with NAFLD (age 56.2 ± 10.0 years; body mass index 37.7 ± 5.3 kg/m^2; 7 women and 5 men) signed informed consent to voluntarily participate in this double-blind, placebo-controlled, crossover trial. Patients had no other chronic diseases besides NAFLD, no history of use of any dietary supplements or drugs within 4 weeks before study commences and were non-pregnant.

Table 1 Baseline characteristics of study participants ($n = 12$).

	Mean ± SD	Min — Max
Age (years)	56.2 ± 10.0	46—70
Weight (kg)	109.2 ± 26.3	79.8—149.4
Body mass index (kg/m^2)	37.7 ± 5.3	32.9—44.6
Body fat (%)	42.8 ± 8.0	28.8—48.5
Waist circumference (cm)	88.7 ± 8.3	77—101
Insulin (IU/mL)	21.4 ± 26.3	7.1—68.1
Leptin (ng/mL)	59.4 ± 26.2	20.2—88.1
Ghrelin (ng/mL)	109.6 ± 41.0	45.8—190.7
AST (U/L)	19.8 ± 6.1	15—29
ALT (U/L)	20.6 ± 6.1	14—30
γ-GT (U/L)	26.8 ± 13.8	14—44
ALP (U/L)	71.4 ± 12.3	55—87
CK (U/L)	120.4 ± 11.6	104—134
Total cholesterol (mmol/L)	5.3 ± 0.9	3.7—6.2
LDL cholesterol ((mmol/L)	3.3 ± 0.9	2.1—4.2
HDL cholesterol (mmol/L)	1.0 ± 0.2	0.9—1.2
Triglycerides (mmol/L)	2.1 ± 1.3	0.9—4.3
Glucose (mmol/L)	8.0 ± 3.9	5.0—13.5

AST: aspartate transaminase; ALT: alanine transaminase; γ-GT: gamma-glutamyl transpeptidase; ALP: alkaline phosphatase; CK: creatine kinase; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

NAFLD was confirmed according to guidelines [4], with all patients had the AST-to-ALT ratio < 2.0 to distinguish NAFLD from alcoholic liver disease. The study was carried out at FSPE Applied Bioenergetics Lab between August and December 2018. The local Institutional Review Board at the University of Novi Sad approved the study protocol (# 0417-HRW/2018). All procedures were performed in accordance with the Declaration of Helsinki, and the trial was registered at ClinicalTrials.gov (NCT03625362). Baseline characteristics of study participants are presented in Table 1.

Experimental protocol

All patients were allocated to receive either 1 L per day of HRW (3 mM of hydrogen) or placebo water (< 0.1 mM

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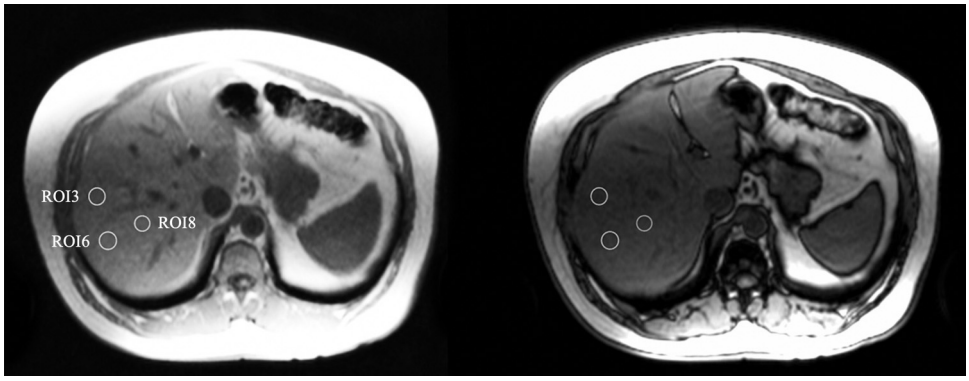

Figure 1 Location of regions of interest (ROI) before (left panel) and after H_2 intervention (right panel). Relatively bright MR signal indicates higher intracellular fat accumulation in the liver.

of hydrogen) for 28 days. HRW was produced by dissolving an effervescent H_2 magnesium-magnesium malate tablet (HRW Rejuvenation) into a cup of lukewarm water (500 mL), through the following reaction: $Mg + 2H_20 \rightarrow 2H_2 + Mg(OH)_2$. Placebo water (500 mL) was produced from tap water, with an introduction of non-hydrogen-producing magnesium tablet, with both HRW and placebo were equivalent for taste, appearance and magnesium amount (80 mg per dosage). Specifically, the placebo tablet produced CO_2 bubbles that mimics bubbles of hydrogen gas in the final drink, making placebo water and HRW similar in appearance for effervescence. The hydrogen concentration in both drinks was measured immediately before consumption (e.g. approximately 5 min after dissolving a tablet in water when no or minimal residue was present) by a gas chromatograph with molecular sieve 5A column and thermal conductivity detector (Hewlett—Packard 5880A, Palo Alto, CA, USA). Patients were instructed to take a drink two times per day before breakfast and dinner. HRW and placebo tablets were provided by HRW Natural Health Products Inc. (New Westminster, BC, Canada). Wash-out period lasted for 14 days in aim to prevent the residual effects of interventions across study periods. The primary treatment outcome was the change in liver fat deposition content as evaluated by magnetic resonance imaging (MRI) from baseline to day 28. Additionally, assessment of body composition outcomes, clinical chemistry biomarkers, and subjective side-effects were performed at baseline and after 28 days after each intervention. Lab assessments were carried out between 08:00 and 10:00 after an overnight fast. MRI was performed using a using a 1.5T system (Avanto; Siemens Healthcare, Erlangen, Germany). Images were acquired with patients in a supine position using an 8 channels body coil. MRI protocol included: tri planar localizer, T2 Weighted coronal, Dual Gradient-Echo In-Phase and Opposed-Phase hepatic MRI and a single-voxel liver ^1H MR spectroscopy. A breath-hold T1-weighted, dual echo gradient echo sequence was obtained in the transverse plane using the following imaging parameters: repetition time 130 ms; echo times (TE) opposed-phase 2.38 ms; TE in-phase 5.04 ms; flip angle 70°; matrix 256×224; slice thickness 6 mm; field of view 300—350 mm. Parallel imaging with a reduction factor of two, was performed in an in-plane, phase-encoding direction. The MR spectroscopic data were acquired with volumes of interest ($2 \times 2 \times 2\,cm^3$) manually placed within the right lobe of the liver, avoiding major blood vessels, intrahepatic bile ducts and the lateral margins of the liver. The point-resolved spectroscopy technique was performed without water suppression (repetition time ms/echo time ms, 2000/35). Spectra were collected following manual shimming, during free breathing. Three regions-of-interest (ROI) were placed in the in-phase magnitude images and transferred to the out-of-phase magnitude images using the copy and paste function of the OsiriX Imaging Software (version 9.5.2, Pixmeo Sarl, Bernex, Switzerland) to ensure perfect co-registration. Care was taken to avoid areas of motion artifacts, large vessels, and bile ducts when placing the ROI. The ROI size was at least $1\,cm^2$. The ROIs were placed in the same liver segments where the MRS is located (Fig. 1). Signal intensity on the in-phase (S_{IP}) and out-phase (S_{OP}) images was measured and calculated the signal fat-fraction using the equation: $\mu = |S_{IP} - S_{OP}|/2\,S_{IP}$ [10]. Metabolite signals were analyzed using the Advanced Magnetic Resonance, or AMARES, fitting algorithm within jMRUI software package [11]. Water peak at 4.76 ppm and the methylene peak at 1.33 ppm were evaluated. Peak integrals were quantified by fitting to a Gaussian line shape. Area ratios (hepatic fat-water ratio) were calculated for each individual.

After initial MRI evaluation, the venous blood was drawn and centrifuged within the next 10 min at 3000 g, with serum separated and analyzed for ghrelin, leptin, and insulin using commercial ELISA kits on an automated analyzer (ChemWell 2910, AWARENESS Technology Inc., Palm City, FL). Serum activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (γ-GT) and creatine kinase (CK) were analyzed by an automated analyzer (Randox Laboratories Ltd., Crumlin, UK). The glucose, total cholesterol, triglycerides, and lipoprotein levels were analyzed by standard enzymatic methods (Hitachi 912, Tokyo, Japan). Height was measured using a stadiometer (Seca 217, Hamburg, Germany), while weight and body fat percentage were measured by a bioelectrical impedance analyzer (Omron BF 511, Kyoto, Japan). Waist and arm circumferences were measured with an anthropometric tape (Gulic CHP, Ann Arbor, MI, USA), and triceps skinfold thickness was obtained with a calibrated caliper (Baseline, Fabrication Enterprises, Inc., White Plains, NY, USA). Arm fat index (AFI) was calcu-

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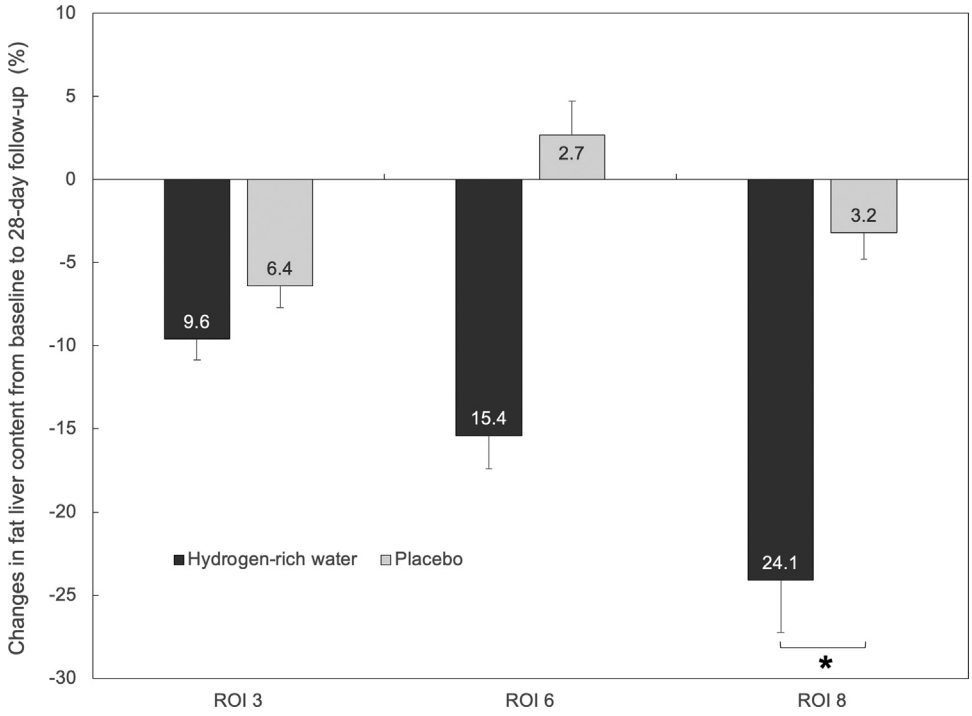

Figure 2 Changes in liver fat accumulation in different regions of interests (ROI). An asterisk (*) indicates significant difference hydrogen-rich water *vs.* placebo at $P < 0.05$.

lated according to the guidelines [12]. The patients were instructed to report on any adverse events of the intervention through an open-ended questionnaire at each visit to the lab. During the trial, the participants were asked to maintain their usual pattern of daily activity and diet.

Statistical analyses

Appropriate sample size ($n = 12$) was calculated using the power analysis (effect size 0.50, alpha error probability 0.05, power 0.80, number of measurements 3, non-sphericity correction ε 1) for the primary treatment outcome (G-Power 3, Heinrich Heine University Düsseldorf, Germany). Two-way mixed model ANOVA with repeated measures was used to establish if any significant differences existed between patients' responses over time of intervention (0 vs. 28 days). When non-homogenous variances were identified, values were compared using Friedman's 2-way ANOVA by ranks. Identification and removal of outliers were conducted according to interquartile range method. The significance level was set at $P \leq 0.05$.

Results

All patients completed the course of the trial, and no participant reported any side effect of either intervention. The compliance with the intervention (as calculated from unused tablets) was 90.0% and 93.3%, for HRW and placebo group, respectively ($P = 0.38$). Dual-echo MRI revealed that HRW significantly reduced liver fat accumulation in individual ROIs at 28-day follow-up, as compared to placebo intake ($P < 0.05$) (Fig. 2).

Baseline total liver fat content was reduced from 284.0 ± 118.1 mM to 256.5 ± 108.3 mM after hydrogen treatment at 28-day follow-up (percent change = 2.9%; 95% confidence interval [CI] from 0.5 to 5.5). Serum AST levels dropped by 10.0% (95% CI; from −23.2 to 3.4) after HRW treatment at the 4-week follow-up. Neither intervention had any significant effects on other liver enzymes ($P > 0.05$), and all values remained in the reference range throughout the trial. Lipid profiles and glucose levels were unaffected by the intervention (not presented). A weak trend has been noted for increased serum insulin levels after HRW administration comparing to placebo (39.5% vs. 20.5%, $P = 0.15$), also for decreased serum leptin (10.5% vs. 31.7%; $P = 0.11$), and increased serum ghrelin at follow-up (96.1% vs. 34.7%; $P = 0.21$). No significant differences were observed between treatment groups in weight, body mass index and body composition indices among participants receiving HRW and placebo ($P > 0.05$) (Fig. 3).

Discussion

In this randomized controlled pilot trial, we found that medium-term supplementation with HRW significantly attenuated liver fat deposition in NAFLD patients, with experimental treatment induced no adverse events. In addition, HRW intervention reduced serum levels of AST, a surrogate biomarker of liver function, while the levels remained in the reference ranges throughout the trial. We controlled the interventions for magnesium content, to account for possible therapeutic effects of Mg on fat liver accumulation [13,14], thus showing for the first time that hydrogen gas per se had a unique beneficial effect in NAFLD. Although preliminary, our trial perhaps nominates HRW as

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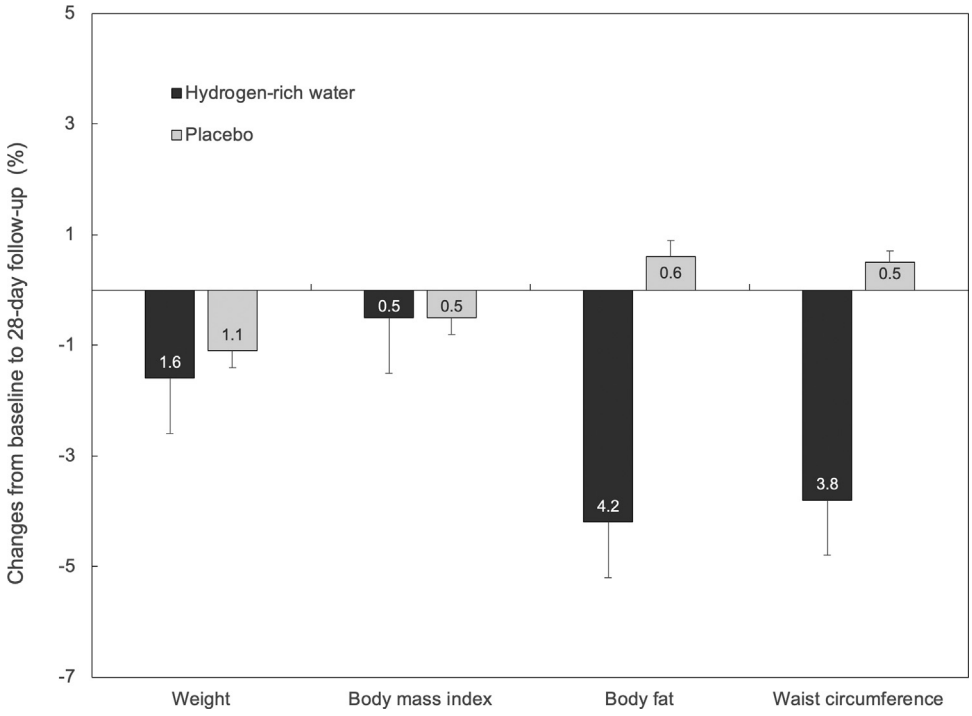

Figure 3 Changes in body composition indices during the study.

a possible adjuvant treatment for non-alcoholic fatty liver disease.

Over the past decade, several animal trials have demonstrated beneficial effects of HRW on liver health and viability. HRW prevented progression of nonalcoholic steatohepatitis and accompanying hepatocarcinogenesis in mice [15]. Koyama and co-workers [9] found that oral intake of hydrogen water significantly suppressed liver fibrogenesis in mice with experimentally induced liver fibrosis. HRW also facilitated hepatocyte mitosis to promote liver regeneration in mice with acetaminophen-induced hepatotoxicity [16]. Another Japanese trial demonstrated that pre-administration of HRW suppresses lipopolysaccharide-induced liver injury in mice [17]. In the most comprehensive study so far, Lin and co-workers [18] investigated the effects of HRW on prevention of early steatohepatitis in mice. HRW treatment significantly reduced ethanol-induced increases in serum ALT, AST, triglycerol and total cholesterol levels, hepatic lipid accumulation and inflammatory cytokines, including tumor necrosis factor-alpha and interleukin-6. HRW also attenuated malondialdehyde level, restored glutathione depletion and increased superoxide dismutase, glutathione peroxidase and catalase activities in the liver. Our study corroborated the beneficial effects of HRW on liver health, with 28-day administration of HRW diminished total fat concentration in the liver (for up to 5.5%) in overweight patients with NAFLD. It appears that fat accumulation dropped throughout the organ, with the most notable reduction seen in the pericapsular area (ROI8), with a relative drop of the fat signal on dual-echo MRI ~ 25% on average. This was accompanied by a drop in serum AST levels, perhaps indicating reduced hepatocellular injury at post-administration. HRW appears to protect hepatocytes from damage by triggering adaptive responses against oxidative stress-related with liver damage [18], including upregulation of specific hepatic oxidoreduction-related genes [19]. Previous human studies have shown that HRW also improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance [6], and reduces body fatness (and advances insulin response) in overweight women [5]. A possible mechanism by which HRW controls lipid metabolism includes improved insulin sensitivity by inducing hepatic fibroblast growth factor 21 that consequently leads to better fuel utilization and less accumulation of body fat [8]. Above favorable outcomes of HRW for liver fat were not accompanied by changes in lipid profiles, serum hormones and body composition in our cohort of NAFLD patients, although we demonstrated a trend for HRW-driven insulin rise. The lack of HRW effects on metabolic profiles in NAFLD might be due to a lower dosage of hydrogen used in our study or shorter duration of treatment, with previous trials used ~ 6 ppm of H_2 per day [5], or administered HRW for 8 weeks [6].

Although this is the first human study describing HRW effectiveness and safety in NAFLD, several limitations must be considered when study findings are interpreted. Relatively small cohort allowed no subsample analysis for possible gender differences in response to HRW intervention in NAFLD. A rather short duration of HRW treatment prevented any unambiguous presumption on the long-term safety of HRW. A limited number of parameters assessed hampered final conclusions on HRW metabolic behavior in NAFLD and its possible mechanisms of action. In addition, we recruited here a population of patients with mild-to-moderate NAFLD while patients with severe NAFLD might respond differently to HRW intervention. Finally, advanced techniques to evaluate more discrete changes in liver histology (including liver needle biopsy or multiparametric MRI)

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should be performed in future studies in terms of utility and comparative effectiveness of HRW in NAFLD.

Conclusions

In summary, a 28-day oral administration of HRW (1 L per day) markedly reduced liver fat content and improved liver enzyme profile for AST in a cohort of overweight patients with NAFLD. Oral hydrogen appears to be a safe supplement that may be proposed as an adjuvant treatment in this population. These observations provide a rationale for further clinical trials to establish the safety and efficacy of H_2 in NAFLD.

Author contributions

SMO conceived and designed research. DK, VS, and JO collected the data. DK, VS, JO, TL, and SMO analyzed and interpreted the data. SMO drafted the manuscript. DK, VS, JO, and TL performed a critical revision of the manuscript.

Disclosure of interest

The authors declare that they have no competing interest.

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