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EFFECT OF COVI-SOUP ON CD4 CELL COUNT AND HAEMOGLOBIN AMONG PATIENTS WITH DIABETES TYPE II AND HYPERTENSION IN KAKAMEGA COUNTY, KENYA: A RANDOMIZED CONTROL TRIAL

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ABSTRACT

Type II diabetes mellitus (DM) and hypertension (HTN) diseases are part of the world's challenges in achieving sustainable development goals. Cases of type II diabetes mellitus and hypertension diseases in low and middle-income countries are becoming a concern. Type II diabetes mellitus is an inflammatory disease. When there is inflammation the immune system is triggered and haemoglobin level is affected. While there have been numerous strategies to address the issue, these efforts have not been sufficient to guarantee a reduction in disease severity. This was an experimental study conducted in Kakamega County, Kenya. It employed a randomized control trial approach, with two groups: treatment and control. Systematic random sampling was used in identifying participants and simple randomization was used to allocate the respondent to the intervention groups. Sample sizes of 34 (treatment group) and 30 (control group) were used to produce quantitative data. This study evaluated the effect of COVI-soup on haemoglobin (Hb) and cluster of differentiation 4 (CD4) cell count of patients living with both type II diabetes mellitus and hypertension. The treatment group received COVI-soup (a soup composed of butternut squash, ginger, garlic, sunflower pepper, chia seeds and turmeric) while the control group was on a rice soup. Statistical Packages for Social Science (SPSS) version 20 was used in the data analysis. A paired student t-test was used to determine the effect of COVI-soup on Hb and CD4 cell count. There was a positive effect in CD4 cell count in the treatment group (P =0.001) whereas there was no significant effect in the control group (P =0.267). The mean change in CD4 cell count in the treatment group was 97 cells/ul and in the control 75 cells/ul. There was a positive effect (increase) on hemoglobin levels in the treatment group (p=0.0001) whereas there was no significant effect in the control group (p =0.126). The hemoglobin mean change in the treatment group was 3.9 g/dl and in the control 0.5 g/dl. Therefore, COVI-soup had a positive effect on hemoglobin and CD4 cell count levels among patients with both hypertension and type II diabetes mellitus.

Key words: Hemoglobin, CD4 cell count, COVI-soup, Rice soup, diabetes type II, hypertension

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INTRODUCTION

The World Health Organization (WHO) classified type II diabetes mellitus (DM) and hypertension (HTN) as factors that increase vulnerability to contracting Coronavirus disease 2019 (COVID-19) [1]. The two diseases have thus gained a lot of attention globally. Type II diabetes mellitus is the 4th leading cause of deaths in the world and hypertension accounts for 7.6 million deaths per annum worldwide [1]. According to the International Diabetes Mellitus Federation there are approximately 463 million diabetics and 769 million people with hypertension globally [2]. The prevalence rate of hypertension in Africa is at 48%, and type II diabetes mellitus is at 5.1% [3]. In Kenya, the prevalence of type II diabetes mellitus is at 4.6 % and hypertension at 29% [4]. Kakamega County has a prevalence rate of 2.8% for type II diabetes mellitus and 8.3% for hypertension [4].

For a long time, type II diabetes mellitus has been considered as a disease of the high income countries; however, the trend has changed and now majority of patients with type II diabetes mellitus are living in middle and low-income countries [5]. The pattern of disease in Africa is shifting, and chronic diseases are on the rise [6]. In sub-Saharan Africa, type II diabetes mellitus is a major cause of death and disability-adjusted life years. In 2018, 17,733 Kenyans died of type II diabetes mellitus and diabetes mellitus-related complications. Health promotion is a key component in managing and preventing type II diabetes mellitus [8].

In sub-Saharan Africa and other parts of the world, there is no question that lifestyle changes have had negative health consequences. Type II diabetes mellitus and hypertension are becoming more common in sub-Saharan Africa due to the high frequency of possible risk factors such as obesity, rapid urbanization, physical inactivity, aging, nutrition transitions, and socioeconomic changes [1]. Although the prevention and control of diseases such as hypertension and type II diabetes mellitus has gained traction in most sub-Saharan African countries in recent years, much more work need to be done to reduce the region's hypertension and diabetes burden [7]. Strategies against type II diabetes mellitus and hypertension should be sharpened through comprehensive approaches which include various sectors beyond health [9]. Type II diabetes mellitus and hypertension coexist in 60 to 70 % of patients with type II diabetes mellitus [7]. This makes it necessary to study both type II diabetes mellitus and hypertension.

In recent years, there has been a remarkable increase in understanding of the involvement of T cells in the pathogenesis of type II diabetes mellitus [5]. Cluster of differentiation 4 (CD4) helper T cells also play an important role in a number of

type II diabetes mellitus related problems. In patients with type II diabetes mellitus, the number of activated T lymphocytes and inflammatory cytokines increased in the kidneys [10]. The activation of Th2 cell-mediated immunity is delayed and hindered in diabetes, according to experimental studies [5]. The circulating levels of Th1-associated cytokines were shown to be higher in diabetes patients [10]. T cell-related cytokines including IL-10 and IL-17 were found to be considerably greater in type II diabetes mellitus and hypertensive patients, implying that T cells are involved in diabetes [5]. It is clear that innate and adaptive immune systems are involved in both type II diabetes mellitus and hypertension, hence they are classified as autoimmune illnesses [11].

Hot pepper aids in the production of blood by increasing iron absorption [12]. Turmeric's carination action helps to boost immunity. Traditional medicine has used it to treat stomach pain and distension, as well as to increase appetite and liver function. Turmeric has been shown to aid in weight gain [13]. However, an Indonesian study discovered a significant increase in haemoglobin levels in broilers fed turmeric [14]. The tiny sunflower seed contains unsaturated fats, fiber, and other crucial micronutrients such phytochemicals, selenium, copper, folate, iron, vitamin E, zinc and vitamins [15]. Oilseed crops have recently received attention due to their beneficial effects on illness prevention and health promotion [16]. The beta carotene concentration of butternut squash is high. It is clear that adding butternut squash flour to a product improves its nutritional content.

The use of dietary modification in managing type II diabetes mellitus has been in existence; however, there is need for complementary intervention so as to guarantee impact. Functional foods have shown to be effective in management of other conditions such as cancer, and thus there is need to translate the use of functional foods in management of type II diabetes mellitus. Dietary active compounds (functional foods) vitamins, probiotics, bioactive peptides, phytochemicals and antioxidants are increasingly being used in disease management [17]. COVI-soup is a nutrient-dense soup made with butternut squash, ginger, garlic, turmeric, chia seeds, sunflower seeds and pepper. COVI-soup ingredients contain phytochemicals that are key in management of type II DM and HTN. A combination of these ingredients enhances nutrition composition and nutrient density hence, increasing efficacy in disease management. COVI-soup ingredients individually have been used in management of type II diabetes mellitus although there is limited information on appropriate combination of these ingredients that have been documented. The aim of the study was to establish the effect of combined ingredients (ginger, garlic, turmeric, chia seeds, sunflower seeds and pepper (COVI-soup) on CD4 cell count and Hb.

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MATERIALS AND METHODS

This was a parallel double-blinded randomized control clinical trial which had two arms: one group was the control, and the other treatment. The control group was administered rice soup while the treatment group patients received COVI-soup. The study participants received a daily ration for three months and the primary outcomes HB and CD4 cell count.

Study setting

This research was carried out at the diabetic clinic in the Kakamega County Teaching and Referral Hospital.

Study participants' recruitment, inclusion and exclusion criteria

The study was done among patients aged between 30 and 70 years, with both type II diabetes mellitus and hypertension attending clinic at Kakamega County Teaching and Referral hospital, and on antihypertensive and antidiabetic drugs (Calcium Channel Blocker/ thiazide+ Angiotensin Converting Enzyme Inhibitor / Angiotensin Receptor Blocker) and on metformin for type II diabetes mellitus with a duration of illness of less than 5 years. Consent was sought from all participants. Patients who did not give consent, critically ill, pregnant mothers and patients with other co-morbidities other than diabetes type II and hypertension were excluded. Patients from same household who met the inclusion criteria were randomized to receive same intervention to avoid contamination that might be caused by sharing or confusing the food ration.

Randomization, Blinding and allocation of concealment

Randomization of the respondents to either control or treatment groups was done by employing simple randomization using computer generated list of number. This was done using excel by a researcher not involved in the trial. This was a double blinded study where the participant, research assistants and all outcome assessors were unaware of the product the participant received until after the study. To achieve blinding, similar packaging for the two rations and secret serial numbers were assigned to both COVI-soup and rice soup. The colour of the soup was also made similar using synthetically acceptable food colours. However, the blinding ended after assessment of the outcomes. Allocation of concealment was done using sequentially numbered opaque sealed envelopes. The list of random numbers was kept away from the research team that distributed food.

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Intervention products and participant visits

COVI-soup and rice soup provided 62 kilocalories each per serving. All the ingredients were bought from the local market and washed before and after peeling to remove all the dirt. The peeled ingredients were sliced thinly with a manual chipper. They were dried using oven dryers to a moisture content of 9%, and then milled to pass through a fine mesh used for commercial maize milling. After grinding, various ingredients were measured at specific amounts. The products were then mixed, then packed using sterile material into one serving. One serving contained 16.35 grams of the enriched soup powder. The various ingredients were measured and mixed in different ratios. This was done 9 times while checking on the palatability of the soup. The ratios for the most palatable soup were as follows; butternut squash 3.75 grams, ginger 0.75 grams, garlic 0.5grams, sunflower seeds 0.5 grams, pepper 0.1grams, chia seeds 0.5 grams, thickening agent 10 grams, and turmeric 0.25 grams.

COVI-soup contained additional medicinal and therapeutic properties (as shown in the introduction) whereas rice soup did not. They were all packed in identical food packaging and labelled with computer generated barcodes corresponding to COVI-soup and rice soup by a third party not involved in the study. Patients who agreed to participate in the study were divided into two groups: treatment and control. The treatment group was given COVI-soup and the control group was given rice soup. Each participant received a daily ration of one sachet that contained 16.35 of either COVI-soup or rice soup depending on the group they were assigned for three weeks, or 21 sachets. After the 21 days, the respondents returned, and monitoring was done on the preparation methods, adverse effects and treatment contamination. COVI-soup and rice soup were thereafter administered for three more weeks, followed by the rest of the three-month research period. The soup was consumed at any time of the day so long as one whole sachet was taken within the day. A demonstration on the soup preparation was done for all the study participants. The trial lasted three months, and each participant received one serving of COVI-soup or one serving of rice soup every day.

CD4 cell count measurement

CD4 cell count analysis was done by a trained laboratory technologist using BD FacsPresto machine. During the incubation time, particular antibodies bind to surface antigens on T-lymphocytes and monocytes when blood is injected into the BD FacsPresto cartridge. When the stained cartridge is introduced into the counter, the software recognizes and counts the CD4+ T-lymphocytes in absolute and percentage cells, as well as calculating the hemoglobin concentration. As a quality control measure, the BD FacsPresto cartridge contains immobilized

antibodies, which the instrument utilizes to check that the reagents are present and that enough blood specimen volume has been added. After blood samples were taken from the patient, they were stored in green top stabilizer tubes. Laboratory personnel who helped in this study were trained and employed at the Kakamega County Referral Hospital. To protect against exposure to blood borne pathogens, gloves were worn when handling participant specimens. Drawing, receiving, storing and redirecting specimens were the responsibility of laboratory workers when necessary. The laboratory staff ensured samples during collection contained visit Date, participant ID, date and time of specimen collection. Two millilitres of blood was collected for analysis and stored in stabilizer tubes.

Compliance to the intervention

The degree of sharing of the assigned intervention/treatment with other household members was also assessed by interviewing the patients at the hospital during the revisits. The patients were asked to come with empty packets of the intervention products during revisits. To improve adherence to the preparation protocol and consumption among participants, phone calls and (sms reminders) messages were sent twice weekly. However, in order to improve adherence, extra information and tactics was required. Face-to-face appointments with the physician and nutritionist were also used. Additionally, a chart on patient adherence was placed at the clinic to motivate the patients. This helped in getting quality and honest feedback from the participants, hence enhancing adherence measures. The research participants were advised not to share the rations given. Since this was a ration that was taken daily and was taken at home it was hard measuring the adherence of not sharing the rations. This was instead advice to the patients. This was meant to support other compliance methods such as phone calls and clinic appointments

Sample size determination

The sample size was adopted from a previous study that used Level of significance = 5%, Power = 80%, Zα = Z is constant set by convention according to accepted α error and Z (1-β) = Z is constant set by convention according to power of study.

$n = 2 (Z\alpha + Z [1-\beta])^2 \times SD^2 / d^2$

$Z\alpha = 1.96$, $Z (1-\beta) = 1.28$, $SD = 15$, d (effect size) $= 20$

So $n = 2 (1.96 + 1.28)^2 \times 13^2 / 10^2 = 35$

35 individuals in each group.

$n = 35*2 = 70$

Data analysis

Normality test was done using Kolmogorov-Smirnov normality test. Frequencies, percentages and standard deviations were analysed using SPSS version 20.

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Primary outcomes were changes in CD4 cell count and Hb level. Paired t test was used to test the effect of the intervention products on the primary variables.

Acceptability of COVI-soup

A total of 30 adults of various ages and genders were randomly selected from the diabetic clinic at Kakamega County Teaching and Referral Hospital. Small amounts of soup were placed in mugs. Each patient evaluated the soup on their own and recorded their ratings on a data sheet. The products were evaluated for sensory acceptability using a five-point hedonic scale (1 = very bad, 2 = bad, 3 = neither good nor bad {borderline}, 4 = good, 5 = very good). An average of all the results was calculated, and an average of 4.1 was discovered across various sensory tests. COVI-soup thus passed the acceptability test.

Ethical considerations

This study was conducted according to the guidelines laid down in the Declaration of Helsinki [18]. All procedures involving human subjects/patients were approved by Masinde Muliro University of Science and Technology's Institutional Ethics Research Committee with reference number (MMUST/IERC/026/2021), and the National Commission for Science, Technology, and Innovation (NACOSTI) with reference number (License No: NACOSTI/P/21/14402) to conduct the study. Written or verbal informed consent was obtained from all patients. Verbal consent was witnessed and formally recorded. Ethical principles on justice, autonomy, beneficence and confidentiality were adhered to. The study was also registered with Pan African Clinical Trial Registry (PACTR) with reference number (PACTR202109794022406) and can be accessed from https://pactr.samrc.ac.za/TrialDisplay.aspx?TrialID=16062

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Study Profile

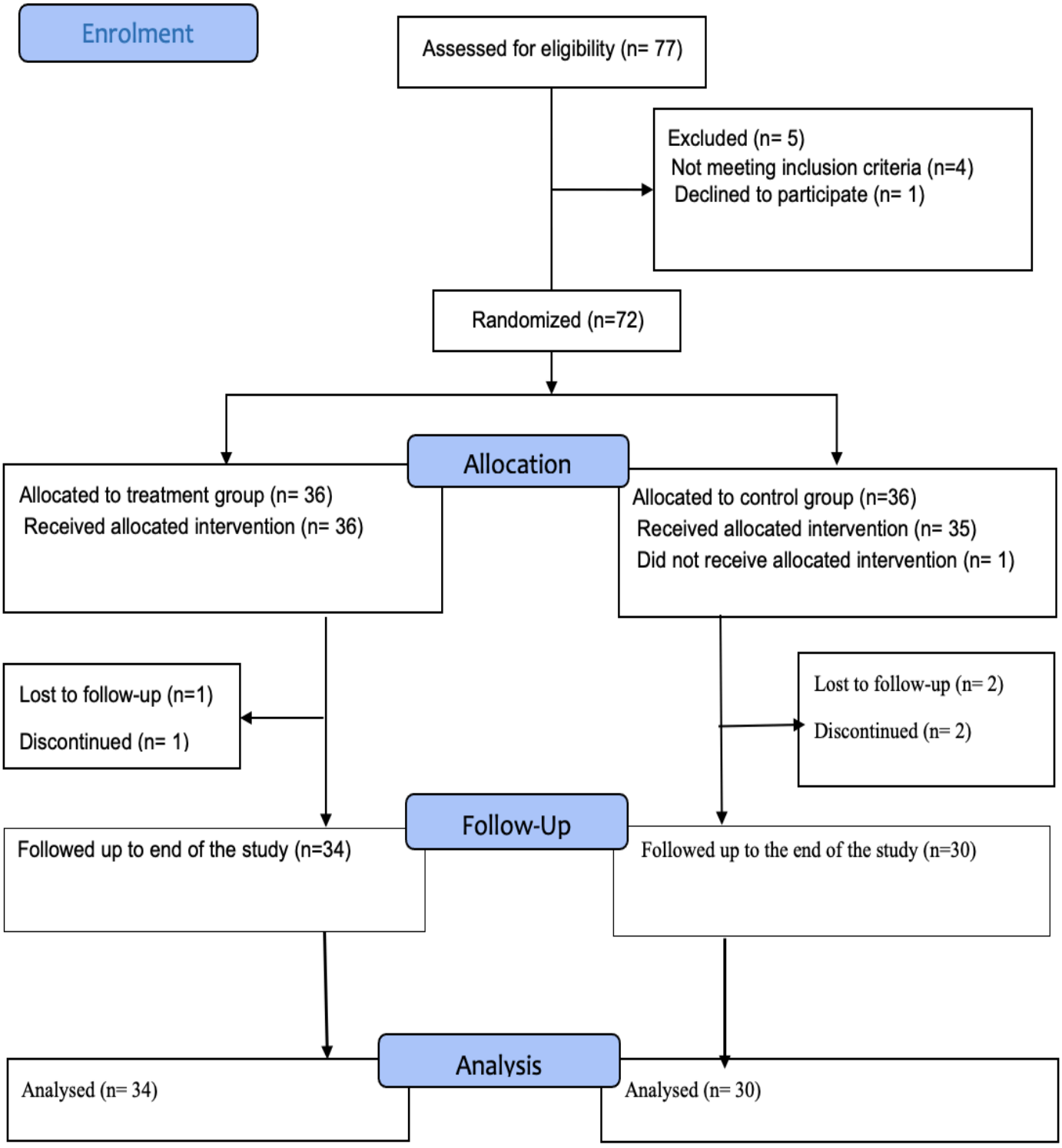

Figure 1: Consort 2010 flow diagram

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RESULTS AND DISCUSSION

Sociodemographic characteristics form a basic part in disease prognosis and recovery.

The current study revealed that in the treatment group, majority of the respondents were female 73.5% (n=25) and male were 26.5% (n=9), whereas in the control group men were the majority at 60.0% (n= 18) and female were 40.0% (n=12). Table 1 below shows the level of education of the respondents. Few respondents in the treatment group had no education 11.8% (n=4). A larger number of the respondents in both groups only attained primary education with treatment representing 44.1% (n=15) and control 40.0% (n=12). Education is a crucial component in the management of type II diabetes mellitus and hypertension. This is because it affects decisions in regard to food choices [19]. However, in this study majority of the respondents had not attained tertiary level of education. In the treatment group, a larger number of the respondents were small-scale traders representing 35.3% (n=12), those who were not employed were 17.6% (n=46), those employed were 14.7% (n=5) of the respondents and those doing casual labor were 32.4% (n=11). In the control group, majority of the respondents were also small-scale traders/ farmers representing 50.0% (n=15) of the respondents. Casual laborers, employed with salary and those not employed each represented 16.7% (n=5) of the respondents. In the treatment group, most of the respondents were aged 61-70 years, 38.2% (n=13) whereas in the control group, most of the respondents were aged 41-50 years, 43.3% (n=13). In both groups, the least number of respondents were aged 30-40 years.

The mean CD4 cell count for the treatment group at baseline was 975 cells/ul and at follow-up it was 1072 cells/ul as shown in Table 3. In the control group, the mean CD4 cell count at baseline was 969 cells/ul and 896 cells/ul after the intervention. In the treatment group, there was a significant change in CD4 cell count (P =<0.001) and in the control there was no significant effect in CD4 cell count (P =0.267). The mean change in the treatment group was 97 cells/ul and in the control 75 cells/ul. Cluster of Differentiation 4 (CD4) plays an important role in immunity of persons with type II diabetes and hypertension. The paired student t-test proved that there was no significant difference between CD4 cell count values of the control group. This means that the participants did not experience a significant change in CD4 cell count, having received the rice soup. In the treatment group paired t-test proved that there was a significant difference between the CD4 cell count values. This means that the participants experienced a change in CD4 cell count having received the COVI-soup. Garlic is one of the most

employed seasonings for cooking. The trend toward the use of natural remedies with fewer side effects has given rise to garlic consumption as an alternative therapy for diseases such as hypertension [17]. According to a previous study, garlic has been shown to improve insulin sensitivity and associated metabolic syndromes hence, helping in regulation of blood glucose [20]. Curcumin has been shown to enhance transcription of several insulin responsive genes and improve insulin resistance on patients with type II diabetes mellitus [21]. Piperine has shown to extensively enhance bioavailability and promoting absorption of drugs and nutrients [22]. Pepper has also been shown to help in glycaemic control by inhibiting hepatic glycogenesis [23]. Ginger enhances synthesis of hepatic glycogen through the enhancement of glycogen regulation enzymes expression in the liver, inhibition of carbohydrate metabolizing enzymes, stimulation of the pancreas to release insulin and inhibition of hepatic glucose [24]

According to Table 3, for the treatment group at baseline, the mean hemoglobin was 11.5g/dl and at follow-up, it was 15.4 g/dl. In the control group the mean hemoglobin level at baseline was 12.1 g/dl and 11.6 g/dl in follow-up. In the treatment group, there was a significant change in hemoglobin level (P =<0.001) and in the control there was no significant effect on hemoglobin (P =0.0126). The mean change in the treatment group was 3.9 g/dl and in the control 0.5 g/dl. The paired t-test proved that there was no significant difference between HB count values of the control group. This means that the rice soup does not have an effect on HB. The paired t-test proved that there was a significant difference between the HB count values of the treatment group. This means that COVI-soup has an effect on HB.

Contrary to this study, a study on ginger supplementation significantly reduced the levels of HbA1c [24]. The study reported that compounds of ginger such as 6-gingerol, tannins, polyphenolic compounds, flavonoids, and triterpenoids possess hypoglycaemic and other pharmacological properties. The study by Hayat *et al.* [25], also suggested that ginger, via its major component, gingerol, by inhibition of key enzymes relevant to type II diabetes mellitus, α-glucosidase and α-amylase, are known enhance management of type II diabetes mellitus. Findings of this study were in agreement with findings where garlic had a significant positive effect on HB among the treatment group [25].

Limitation of the study

Simple randomization was used in this study and this affected the balance of participants in both groups in terms of age, sex, occupation and educational status.

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The study did not adjust for variability in study group characteristics, therefore, the results should be interpreted with caution.

CONCLUSION

This study found that combining the following ingredients had a significant effect on hemoglobin and CD4 cell count levels: ginger, garlic, turmeric, hot pepper, chia seeds, sunflower seeds, and butternut squash. As a result, COVI-soup has a positive effect (an increase in hemoglobin and CD4 cell count levels) on patients with both hypertension and type II diabetes mellitus. The study recommends that:

1. Further research should be conducted on COVI-soup on a larger population of more than 150 participants in each group so as to enhance study power and control for confounders, using multivariate analysis.
2. Further research should be conducted on the effect of COVI-soup on other biomarkers like HBA1c and lipid profiles in patients with type II diabetes mellitus.

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CONFLICT OF INTEREST

None of the authors declared conflict of interest.

CONTRIBUTORS

The authors' responsibilities were as follows: SC, JS, and CW conceived the idea and designed the study. SC and AK trained the research assistant. BW supervised data collection. JS, SC, AK and CW developed COVI-soup. SC analysed the data and drafted the manuscript. CM, JS, LM, RO, BW and CW offered supervisory and overall editorial oversight.

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Table 1: Sociodemographic characteristics of the respondents

Sociodemographic characteristics		Treatment group n (%) n=34	Control group n (%) n=30	P value
Age	30- 40 years	1 (2.9)	2 (6.7)	0.89
	41-50 years	8 (23.5)	13 (43.3)	
	51- 60 years	12 (35.3)	11 (36.7)	
	61-70 years	13 (38.2)	4 (13.3)	
Education	No education	4 (11.8)	5 (16.7)	0.62
	Primary	15 (44.1)	12 (40.0)	
	Secondary	9 (26.5)	8 (26.7)	
	Tertiary	6 (17.6)	5 (16.7)	
Gender	Female	25 (73.5)	12 (40.0)	0.56
	Male	9 (26.5)	18 (60.0)	
Duration of illness in years	1 -2 years	10 (29.5)	9 (30.0)	0.72
	3-5 years	24 (70.5)	21 (70.0)	
Occupation of the respondent	Employed with a salary	5 (14.7)	5 (16.7)	0.56
	Small scale trading	12 (35.3)	15 (50.0)	
	Casual labour	11 (32.4)	5 (16.7)	
	Not employed	6 (17.6)	5 (16.7)	

Data are presented as frequency and percentage as indicated. Statistical analysis was performed using chi-square and Fisher's exact test for gender distribution. Data on age, education, gender, duration of illness and occupation of the respondents are presented by categories under each variable. Age and duration of illness are presented in years. The significant P values are in bold

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Table 2: Baseline characteristics of study participants per intervention

	Control group	Treatment group	P value
CD4 cell count (cells/ul)	969±294	975±261	0.298
Haemoglobin level (g/dl)	12.1±0.9	11.5±1.2	0.391

Data are presented as means and standard deviation as indicated. Statistical analysis was performed using student t test. This table presents data on, CD4 cell count and haemoglobin level for both the treatment and control groups before the interventions. The significant P values are in bold

Table 3: Characteristics of the respondents after intervention

Variable		Before	After	Changes	P value
CD4 cell count (cells/ul)	Control	969± 294	894± 215	75	0.267
	Treatment	975± 261	1072± 260	97	**0.001**
Haemoglobin (g/dl)	Control	12.1± 0.9	11.6± 1.2	0.5	0.0126
	Treatment	11.5± 1.2	15.4± 1.1	3.9	**0.0001**

Data are presented as means, mean change and standard deviation as indicated. This table presents data on CD4 cell count and haemoglobin level for both the treatment and control groups before and after the interventions. The significant P values are in bold

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