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Effect of Dietary Supplementation of Maize (*Zea mays*) with Soybean (*Glycine max*) and Moringa (*Moringa oleifera*) on Blood Biochemical Profile of Albino Rats

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Authors' contributions

This study was carried out in collaboration between all authors. Authors BAI and AYK designed the study and all the necessary while statistical analysis, writing and editing the manuscript were jointly done by all the authors. All authors read and approved the final manuscript.

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ABSTRACT

Effects of supplementation of maize with soybean and Moringa leaf on selected blood parameters in albino rats were investigated. Albino rats weighing 130 – 150 g were grouped into six. Group 1 was fed with normal chow, groups 2 and 3 were fed with maize supplemented with soybeans in ratios 4:1 and 3:2, groups 4 and 5 were fed with maize supplemented with Moringa in ratios 4:1 and 9:1, while, group 6 was fed with maize. At the end of one month study group 4 had the highest reduction in blood glucose and higher increase in albumin by 11.7% and 9.49%. There was decrease in cholesterol in groups 2 and 4. Albino rats fed with Moringa supplementations had the highest HDL increment compared to other groups. All the groups had reduced LDL levels. This study shows that Moringa and soybean supplementation have significant effect on blood glucose, cholesterol, HDL and LDL.

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1. INTRODUCTION

The dearth of information of supplementation of maize with Moringa leaf and soybean was the thrust of this study. Most of the studies on supplementation of cereals with other legumes centred on in vitro approach. This in vivo approach gave the true nature of the contribution of maize supplementation on some blood parameters. So that the health benefits of the supplementation at specific ratios are optimised.

Diet and nutrition are important factors in the promotion and maintenance of good health throughout the entire life course. Their roles as determinants of chronic non-communicable diseases (NCDs) is well established and they therefore, occupy a prominent position in prevention activities [1]. It has been projected that, by 2020, chronic diseases will account for almost three-quarters of all deaths worldwide and that 71% of deaths will be attributed to ischemic heart disease (IHD), 75% of deaths due to stroke, and 70% of deaths due to diabetes and these will be prevalent in developing countries [2]. The number of people in the developing world with diabetes will increase by more than 2.5-fold, from 84 million in 1995 to 228 million in 2025 [3]. On a global basis, 60% of the burden of chronic diseases will occur in developing countries. Indeed, cardiovascular diseases are even now more numerous in India and China than in all the economically developed countries in the world put together [4]. As for overweight and obesity, not only has the current prevalence already reached unprecedented levels, but the rate at which it is annually increasing in most developing regions is substantial [5].

It is therefore important that available and cheaper source of nutritious ad healthy foods be sourced, processed and appropriately utilized. Several local plants can be used as supplements to routine meals so as to provide the needed nutrients. *Moringa* and Soya beans can eventually play this role as they are rich in vast nutrients, minerals and important bioactive compounds. *Moringa olifera* leaves are very nutritious can be consumed in any form. Since dried Moringa leaves retain their nutrient content [6] it is easy to convert them into powder. Moringa leaf powder is an excellent nutritional supplement and can be added to any dish [7]. The nutritional characteristics of the plant may be potentially beneficial to the developing regions of the world where undernourishment is a major concern [8,9].

Soybean seeds have been shown to have numerous health promoting effects mostly attributed to their high nutrients [10] and phytochemical such as isoflavones [11]. Soybean is not only protein rich, but also a good source of minerals (phosphorous, calcium and iron). Proteins foodstuff such as soybean complements cereal proteins thereby providing an ideal source of dietary protein [11]. Isoflavones present in soybean include genistein and daidzein. Soybean proteins and isoflavones have been shown to reduce the risk of cardiovascular diseases by lowering blood pressure, blood cholesterol and triglycerides [12,13].

2. MATERIALS

2.1 Sample Collection

Maize, soybeans and *Moringa oleifera* plants were obtained from Kure Market in Minna, Nigeria in the month of July, 2013.

2.2 Sample Treatment

Maize and soybean were cleaned to remove dirt while *Moringa oleifera* leaf were washed in water and dried under shade for four (4) days. They were separately milled to obtain a homogeneous powder of 6 mm particle size and stored in plastic air-tight containers until required for further use.

2.3 Experimental Animals

Adult Albino rats weighing between 130 – 150 g were used for this study. The rats were obtained from the Animal Farm of the Ibrahim Babangida University, Lapai, Nigeria.

2.4 Management of Experimental Animals

The animals were allowed to acclimatize for 2 weeks under standard laboratory conditions. They were maintained on standard rat growers' mash and potable water was administered *ad libitum.* The animals were handled in strict compliance with International Guidelines as prescribed by the Canadian Council on the Care

and Use of Laboratory Animals in Biomedical Research (1984) as cited by Somali et al. [14]. After the acclimatization period, the rats in the treatment groups were kept to adapt to the supplemented diets seven days before the experiment commenced properly.

2.5 Feed Preparation

The soybean flour (SF) and *Moringa oleifera* leaf powder (MOLP) were separately supplemented into maize flour (MF) in different ratios. Three different ratios were formulated which included 4:1, 3:2 and 9:1. The first was obtained by adding 100 g of either MOLP or SF to 400 g of MF. Consequently, the other was prepared by mixing 200 g of SF to 300 g of MF and 100 g of MOLP to 900 g of MF respectively.

2.6 Experimental Design

A total of 36 adult male and female Albino rats were used for this study which lasted for 4 weeks (one month). The animals were randomly distributed into six groups (groups 1-6) with six rats each. Rats in group one were place on standard commercial chow (control), groups two and three were fed with SF supplemented MF in ratios 4:1 and 3:2 respectively. Groups four and five were fed with MOLP supplemented MF in ratios 4:1 and 9:1 respectively, while, the sixth group was placed on only MF. The soybeans supplemented maize and Moringa supplemented maize are represented by MSB and MM respectively. The rats were allowed access to food and water freely throughout the experimental period after a week (7 days) adaptation period to the various supplemented diets. At the end of the second week, three rats from each group were fasted for about 12 hours and sacrificed under mild chloroform anaesthesia to collect blood sample. Consequently, internal organs (kidney, heart and liver) were rinsed in distilled water and weighed.

3. METHODS

3.1 Blood Sample Collection

Using chloroform as anesthesia, blood was collected from each rat via heart puncture and transferred into plain sample bottles and spun at 3000 rpm to collect the serum portion which was used for biochemical analysis.

3.2 Determination of Biochemical Parameters

Serum lipids; total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol, glucose, albumin were determined using AGAPE diagnostic kit (AGAPE, Switzerland). Each test was carried out according the manual enclosed.

3.3 Statistical Analysis

The results were analysed using analysis of variance (ANOVA) and were presented as the mean value ± SEM (standard error of mean) for the control and experimental rats. Differences among the means for the groups were assessed using the Duncan's Multiple Range Test to determine which mean values were significantly different at ($p<0.05$).

4. RESULTS AND DISCUSSION

The result of the effect of supplemented feed on blood glucose level (Fig. 1), shows that *Moringa oleifera* supplemented feeds (MM4:1 and MM9:1) caused the highest reduction of blood glucose concentration at the end of the feeding exercise. The reduction in glucose level might be as a result of partly weight reduction effect by the plants and Moringa leaves has been proved to possess hypoglycemic effect [15].

Fig. 2 shows an increase in serum albumin in all the experimental groups except the group fed only maize at the end of the experimental period. The decrease in albumin concentration noticed in the maize fed group is expected because of the low protein content of maize as also observed by Alada et al. [16]. He reported that soybean diets produced a slight but significant increase ($P<0.05$) in plasma albumin at 75% soybean concentration. This was not in agreement with the findings in this study where MSB9:1 with lower soybean concentration displayed a significant reduction in albumin concentration compared to MMSB 3:2. This difference may be due to species variety, methods of cultivation, soil type or method of feed preparation. The elevation of albumin concentration in soybean supplemented maize feed as seen in this study is consistent with the reports of Bolarinwa et al. [17] as cited by Alada et al. [16] who had earlier drawn a correlation between plasma protein levels and severity of protein malnutrition. The protein content of *Moringa oleifera* and soybean which is quite high could be responsible for the high levels of the plasma albumin.

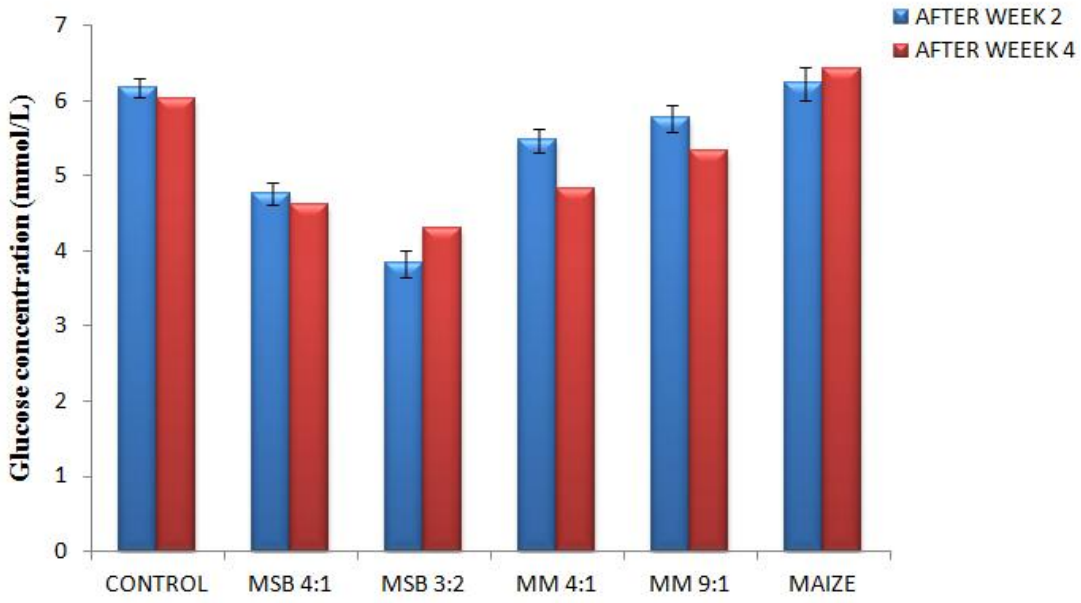

Fig. 1. Effect of supplementation blood glucose concentration of experimental rats

Key: MSB 4:1-Soya beans supplemented maize (4:1); MSB 3:2- Soya beans supplemented maize (3:2); MM 4:1- Moringa supplemented maize (4:1); MM 9:1- Moringa supplemented maize (9:1)

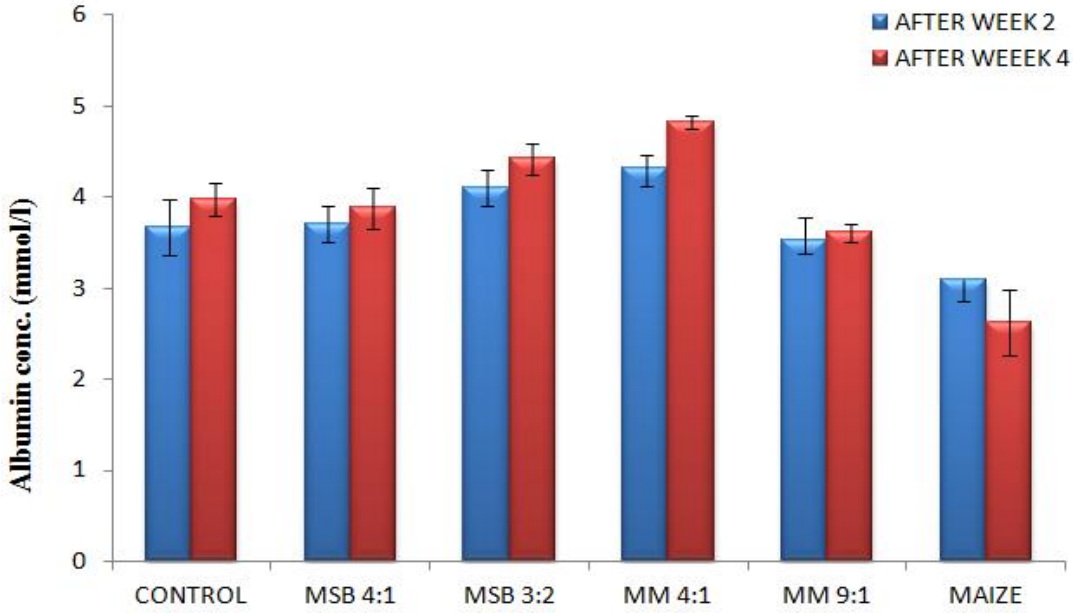

Fig. 2. Effect of supplementation on serum albumin concentration of experimental rats

Key: MSB 4:1-Soya beans supplemented maize (4:1); MSB 3:2- Soya beans supplemented maize (3:2); MM 4:1- Moringa supplemented maize (4:1); MM 9:1- Moringa supplemented maize (9:1)

The effect of the formulated feed on lipid profile shows that only groups placed on MSB4:1 and MM4:1 had slight reduction in cholesterol concentration while others increased slightly but was insignificant (Figs. 3 – 6). The triglyceride concentration decreased in the fed groups except the group placed solely on maize while the LDL concentration reduced in all the fed groups and the control. There was increase in HDL concentration in only the group fed with Moringa-supplemented maize diets (4:1 and 9:1) while the control and soybeans- supplemented

maize groups had reduced HDL-cholesterol concentrations. High plasma HDL-cholesterol represents an increased mobilization of cholesterol from the adipose tissue to the liver where it is metabolized.

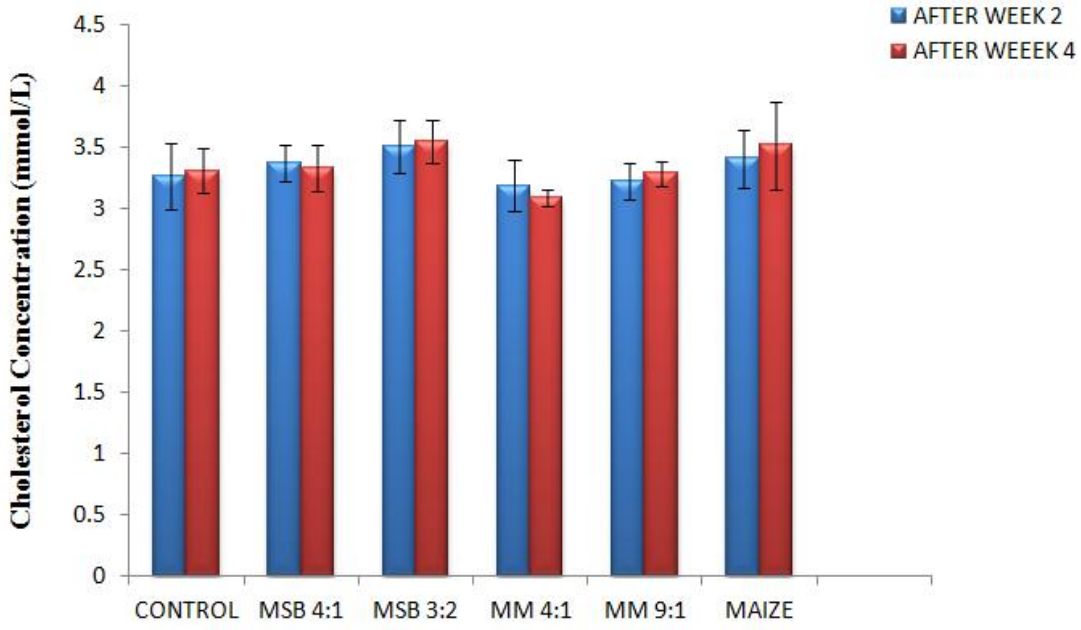

Fig. 3. Effect of supplementation on cholesterol concentration in experimental rats

Key: MSB 4:1-Soya beans supplemented maize (4:1); MSB 3:2- Soya beans supplemented maize (3:2); MM 4:1- Moringa supplemented maize (4:1); MM 9:1- Moringa supplemented maize (9:1)

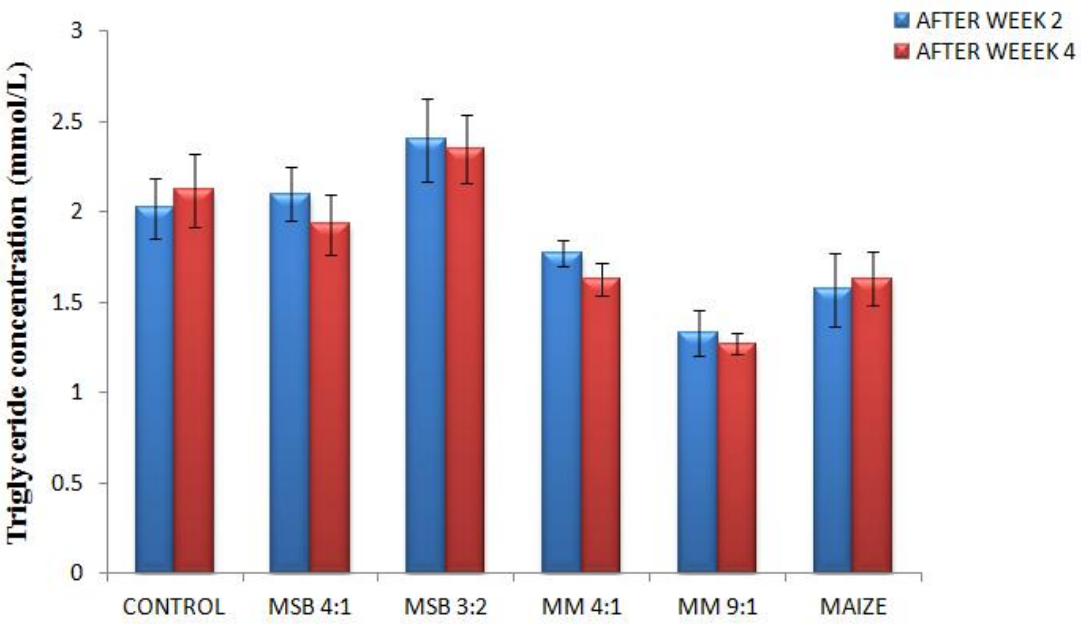

Fig. 4. Effect of supplementation on triglyceride concentration in experimental rats

Key: MSB 4:1-Soya beans supplemented maize (4:1); MSB 3:2- Soya beans supplemented maize (3:2); MM 4:1- Moringa supplemented maize (4:1); MM 9:1- Moringa supplemented maize (9:1)

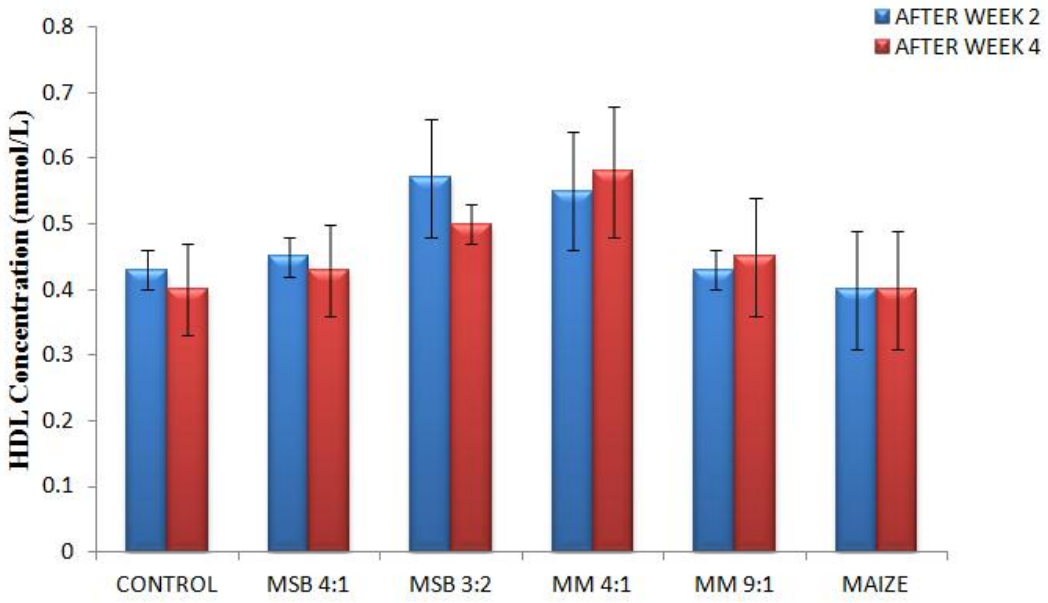

Fig. 5. Effect of supplementation on high density lipoprotein (HDL) concentration in experimental rats

Key: MSB 4:1-Soya beans supplemented maize (4:1); MSB 3:2- Soya beans supplemented maize (3:2); MM 4:1- Moringa supplemented maize (4:1); MM 9:1- Moringa supplemented maize (9:1)

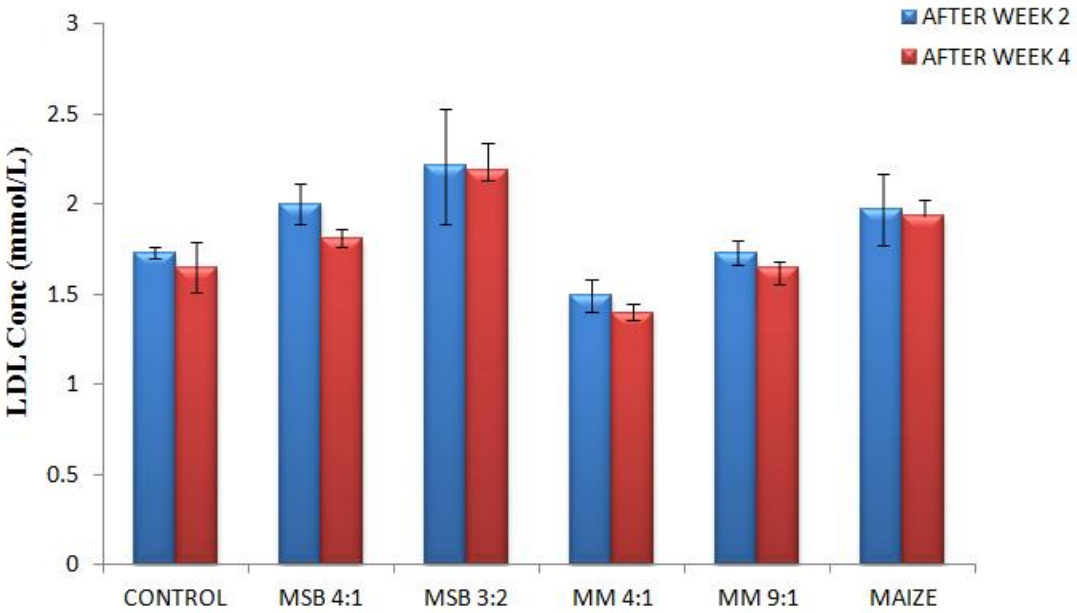

Fig. 6. Effect of supplementation on low density lipoprotein (LDL) concentration in experimental rats

Key: MSB 4:1-Soya beans supplemented maize (4:1); MSB 3:2- Soya beans supplemented maize (3:2); MM 4:1- Moringa supplemented maize (4:1); MM 9:1- Moringa supplemented maize (9:1)

The reduction in the lipid profile as observed in Figs. 3-6 is desirable as it indicates a reduced risk for cardiovascular diseases and hypertension. Studies have unequivocally established an inverse relationship between HDL-cholesterol and incidence of cardiovascular diseases [18,19]. The findings in this work is also supported by the findings of Jain et al. [20], who

reported that the serum cholesterol, triacylglycerides, VLDL, LDL, and atherogenic index were reduced by *Moringa Oleifera* but HDL level was increased as compared to the corresponding high fed cholesterol diet group (control). The increase in HDL and decrease in concentration of cholesterol may have been due to the presence of certain bioactive constituents such as polyphenols which can bind to cholesterol and bile acids, and increase their removal via the faeces. This will therefore result in a decreased cholesterol micelle formation and hence the uptake of lipids from the intestine into the blood is reduced [21]. Saponins are important therapeutically as they have been shown to elicit hypolipidemic effects [22]. Flavonoids have been reported to elicit hypolipidemic effects which may be due to the fact that flavonoids (which could act as antioxidants), could resist LDL oxidation and this could inhibit atherosclerosis [23].

5. CONCLUSION

The results obtained from this study shows that *Moringa oleifera* and soybean supplemented diets are ideal diets for the prevention of malnutrition as well as diseases related to blood glucose, cholesterol LDL, and HDL.

ETHICAL APPROVAL

As per international standard or university standard, ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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