Effects of Lentinula edodes bar on lipid and antioxidant profiles in borderline high cholesterol individuals: A double blind randomized clinical trial

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Protocol

Trial design

A prospective Phase II, simple randomization, double blind and placebo-controlled trial was conducted from September to December 2018. The study was approved by Human Research Ethics Committee from University of Sorocaba (protocol number 2.824.297), in accordance with Resolution 466/2012 of the National Health Council and Research Ethics Review Committee (ERC).

Eligibility Criteria

The disclosure of the clinical study began in May with the formation of a team of collaborators. It was disseminated through folders distributed across all campuses where approximately 10,000 students study. The recruitment was also posted on the University website. Printed material was prepared for distribution so that students or family members could participate. The disclosure containing explanations about the research criteria, inclusion and exclusion happened in social networks aimed at borderline dyslipidemic individuals living in Sorocaba and cities in the region.

For interested individuals, it was essential to answer an online questionnaire with multiple choice questions related to the inclusion and exclusion criteria, which defined the candidate's eligibility. A total of 165 individuals completed the Google online survey.

- Inclusion Criteria

Individuals aged from 20 to 65 years old, of both gender, who had at least one of the following biochemical markers at the borderline high level (total cholesterol 180 to 239 mg/dL; LDL 130 to 159 mg/dL; triglycerides of 150 to 200 mg/dL), were recruited, diagnosed by biochemical exams with dates recent to the day of recruitment. The be tolerant to bars ingredients and to Shiitake. Availability to attend the date and time of blood collection.

- Exclusion Criteria

Some of the individuals had diseases such as cancer, heart disease, neurodegenerative disease, diabetes, among others. These diseases could be confounders of the study and some recruited people had to be excluded. Pregnant, lactating or hormone replacement women could not participate either.

Individuals were instructed to no change their eating habits and patterns, physical activity level or oral contraceptive use during the study, thus identifying only the effects of Shiitake added to the diet for each individual on the exposed group.

Study Interventions.

The composition of the bars is presented in Table 1. Shiitake bar formulation was approved in the sensory analysis in a previous study from our working group. Shiitake concentration of 100 mg/Kg/day showed to be safe in a study conducted with different Shiitake concentrations intake (100, 400 and 800 mg/Kg/day).

Table 1 – Ingredients used in the formulation sweet cereal bars in 25g.

Ingredients	Shiitake Bar		Placebo Bar	
	%	g	%	g
Dry Shiitake	14	3.50	-	-
Flakes oat	12	3.0	23.2	5.80
Brown sugar	8.3	2.10	13.6	3.40
White sugar	30	7.50	20	5.00
Dry plum	9	2.25	10.8	2.70
Brazil nut	7	1.75	8	2.00
Flaxseed	3.2	0.80	3.44	0.86
Glucose	1.6	0.4	1.6	0.40
Soy lecithin	0.4	0.10	0.4	0.10
Quinoa grains	3.5	0.87	4.8	1.20
Peanut	8	2.0	10	2.50
Chia seed	2	0.5	3.2	0.80
Food glycerin	0.5	0.12	0.5	0.12
Coconut oil	0.5	0.12	0.5	0.12
Total	100	25	100	25

Outcomes

The primary outcome of the study was total cholesterol, triglycerides, low-density lipoproteins (LDL) or high-density lipoproteins (HDL) levels. The secondary outcomes included blood glucose, Body Mass Index (BMI) and oxidative stress biomarkers reduced glutathione (GSH), activity of the catalase enzyme and Thiobarbituric acid reactive substances (TBARS).

Sample size

A total of 165 individuals who completed the online questionnaire between May and September 2018, were eligible to participate in this study and were willing to participate and had some borderline dyslipidemia. From this number, individuals who

did not meet the inclusion criteria were excluded. A 95% confidence interval was used for analysis.

Randomization

The individuals (68) were randomly allocated (using a table of random numbers) into 2 groups: I – Placebo group (n = 32) and II – Intervention group (n = 36).

After nutritional assessment and blood collection, the Individuals received an unidentified opaque and sealed, containing bars individually vacuum packed. The bars lasted for a period of 33 days. The patients and the data collector were blinded. The bars of the intervention and placebo groups were similar in texture, flavor, aroma and appearance.

After 33 days (T33), all individuals returned to the University of Sorocaba for new nutritional assessments. They received more food bars and had their blood collected with the same double-blind care. At the end of sixty-six days (T66), subjects returned for the last blood collection and final nutritional assessment.

Blinding

In the blind randomization, a University pharmaceutical and an undergraduate student unrelated to the research to keep full impartiality in the assessment contributed to the distribution of the intervention and placebo using sealed, opaque envelopes which contained specific markings for each group. The volunteers were unaware of which group they were participating in, as the envelopes apparently were the same, only the two external contributors were aware to the marks on the envelopes, because the bars with Shiitake and placebo inside the envelopes had the same size, appearance, aroma and taste.

The researchers did not have access to the identification of participants in the groups, after the distribution of the envelopes, the participants were renamed by the blinded collaborators sequentially numbered from 1 to 68 until the completion of the study.