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Mix of Allegedly Functional Components Improves Metabolic Syndrome Risk Factors

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Abstract

Background: Food is one of the main factors that diminish the risk of development of chronic diseases in humans. In view of this fact, increasing attention has focused on the production and consumption of foods that help reduce this risk. Besides, metabolic syndrome risk factors are increasing in almost all populations of the world. Therefore, the objective of this work was to evaluate the effects of a mixture of ingredients containing textured soy protein, wheat bran, oats, black sesame seeds, white sesame seeds, brown linseed, granola and brown sugar on the biochemical profile, body weight and intestinal motility of Wistar rats.

Methods: The experiment involved male rats, which were divided into two groups: one that ingested the mix and the other a control group.

Results: After 40 days of treatment, it was found that body weight, total cholesterol, triglycerides, and glucose levels were reduced and HDL-c increased. Intestinal transit time was also improved.

Conclusion: It was concluded that the use of this mix had beneficial effects on the metabolic profile contributing to the prevention of metabolic syndrome risk factors and improved intestinal transit of Wistar rats.

Keywords: Fibers; Lipemia; Glycemia; Intestinal motility; Wistar rats

Introduction

The consumption of certain foods has been imputed as one of the main mechanisms for reducing the risk for the development of chronic and degenerative diseases in humans, whose prevalence has been increasing at an alarming rate. Numerous studies indicate that diets rich in fibers and other bioactive components diminish these risk factors, which include insulin resistance and hyperglycemia, high levels of total cholesterol, LDL-c and triglycerides (TG) and reduced levels of HDL-c [1-4].

Dietetic fibers are resistant to digestion and absorption in the small intestine of humans and are classified as soluble (pectin, gum, mucilage and some hemicelluloses) and insoluble (cellulose, lignin and some hemicelluloses) [5]. Evidence indicates that the ingestion of large amounts of dietetic fibers is significantly associated with lower values of body mass index (BMI), systolic and diastolic blood pressure, and serum LDL-c and triglyceride levels [6-9]. Soluble and insoluble fractions also exert beneficial physiological and metabolic effects in the prevention and treatment of disorders such as intestinal constipation and diverticulitis, and inflammatory processes associated with chronic pathological conditions such as metabolic syndrome (MS), obesity, diabetes mellitus type 2 (DM2), cancer, and cardiovascular diseases (CVD) [10-12].

The ingestion of oily grains such as linseed and sesame, which contain high contents of polyunsaturated fatty acids, dietetic fibers, minerals, vitamins and phytoestrogens also has beneficial effects on human health [13-16].

Considering that disorders such as diabetes and obesity have reached global epidemiological proportions and are one of the main causes of death worldwide, particularly due to cardiovascular complications, the optimization of prevention and control strategies related with the consumption of affordable foods has become a pressing concern [17, 18]. In response to this concern, several studies have suggested that the association of fibers and phytochemicals (vitamins, minerals, phenolic compounds and phytoestrogens, which are abundant in grains) is more beneficial to health than the activity of fibers consumed isolatedly [19-23]. Hence, the objective of this study was to supplement the diet of Wistar rats with a product prepared from soy protein, wheat bran, oats, sesame seeds, linseed and granola, which are ingredients widely used by the population, and to assess its effects on the biochemical profile, body weight and intestinal motility of these animals.

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Table 1. Mean and Standard Deviation of the Weight of the Animals of the Control Group (CG) and Mix Group (MG) Before and After the Treatment

	CG		MG		P-value*
	Mean	SD	Mean	SD	
Weight before treatment (g)	224.00	13.98	231.05	11.12	0.8679
Weight after treatment (g)	312.91	44.41	294.46	21.66	0.0254

*P-value < 0.05.

Materials and Methods

Preparation of the mix

The mix of ingredients was prepared in the Food Processing Lab at the Faculty of Technology of Marilia (FATEC), in the state of Sao Paulo, Brazil.

The product was developed from textured soy protein (14.70%), wheat bran (14.70%), oats (14.70%), black sesame seeds (2.94%), white sesame seeds (2.95%), brown linseed (5.90%), granola (14.70%) and brown sugar (29.41%) purchased in the local market. The granola purchased for this study contained oat flakes, barley malt, inverted sugar, wheat germ, crystal sugar, cashew nuts, corn flakes, soy oil, raisins, soy protein and linseed. All these ingredients were mixed (without crushing) and then heated in an industrial oven (FTE 240, G PANIZ) for 15 min at a temperature of 120 °C.

Rat groups

The experiment was approved by the Animal Research Ethics Committee of the University of Marilia (UNIMAR, Marilia, SP, Brazil). Twenty healthy male Wistar rats were used, weighing approximately 230 - 250 g, which were kept in the vivarium at UNIMAR. The rats were housed in collective cages under a dark/light cycle of 12 h, room temperature of 22 ± 2 °C, and relative air humidity of 60±5%. Throughout the experiment, the animals were fed and watered *ad libitum*, and were cared for according to the recommendations of the Canadian Council's "Guide for the care and use of experimental animals".

After a period of 7 days of acclimation to laboratory conditions, the animals were divided randomly into two experimental groups, which were identified according to the food they would receive: 10 animals were treated with the mix (MG) and 10 with commercial rat feed (PURINA®), constituting the control group (CG). The treatment was applied for 40 consecutive days and the animals were weighed at 2-day intervals.

Preparation of the supplemented rat feed

The rat feed supplemented with the aforementioned mix was prepared weekly in the Food Processing Lab at the Faculty of Technology of Marilia, UNIMAR, in a proportion of 40:60 mix/commercial feed.

The commercial feed was crushed manually in a plastic mortar and pestle, after which the mix of experimental ingredients and the commercial feed were ground separately in an electric hammer mill operating at 4,500 rpm (MARCONI). After weighing the two constituents separately on a semi-analytical balance, they were mixed homogeneously, using water as binder. Using a meat-filling machine, this mixture was molded into a cylindrical shape identical to that of the commercial feed. The resulting pellets were dried in an air circulating oven (MARCONI) at 65 °C for about 8 h, stored in polyethylene packaging, and refrigerated until use.

Collection of blood samples and determination of the biochemical profile

After 40 days of treatment and a 10-h fast, the animals were anesthetized with sodium pentobarbital until complete sedation, after which blood samples were drawn from the vena cava to determine their biochemical profile: total cholesterol (TC), HDL-c, triacylglycerides (TG), glycemia, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

The exams were carried out at Clinical Analysis Lab of UNIMAR's Hospital (Marilia, SP, Brazil) and the results were interpreted according to the comparison of the CG.

Table 2. Mean and Standard Deviation of Food Consumption by the Animals of the Control Group (CG) and Mix Group (MG) After the Treatment

	CG		MG		P-value*
	Mean	SD	Mean	SD	
Consumption/day at the beginning of the treatment (g)	11.50	3.2	11.71	2.29	0.0767
Consumption/day at the end of the treatment (g)	13.90	2.9	11.49	1.14	0.0223

*P-value < 0.05.

Table 3. Mean and Standard Deviation of the Biochemical Variables in the Control Group (CG) and Mix Group (MG)

	CG		MG		CG × MG
	Mean	SD	Mean	SD	P-value*
Glycemia	163.20	13.74	141.00	8.21	0.00017
CT	80.00	7.18	50.10	6.95	0.00000
HDL-c	17.10	1.20	28.40	5.74	0.00000
TG	104.90	18.28	60.70	9.50	0.00000
AST	153.00	15.19	125.10	15.29	0.00034
ALT	76.10	16.57	60.00	8.22	0.00654

*P-value < 0.05.

Intestinal motility test

The intestinal motility test was evaluated according to the model described by Michelin and Salgado [24], with modifications. After a 24-h fast, the CG and MG were gavage-fed, respectively, 0.3 mL of saline and 0.3 mL of the ground cereal mix dissolved in water (0.3 g/mL). Then, 45 min later, the two groups were gavage-fed 0.3 mL of a 10% activated charcoal suspension in 5% gum arabic. Four hours after the administration of activated charcoal, the animals were euthanized with a lethal intraperitoneal injection of 200 mg/kg of thiopental. After death was confirmed, the intestines were removed, pinned to a styrofoam board, their length and the distance traveled by the activated charcoal in the intestines of each group were measured with a tape measure.

Analysis of the percent composition

The rat feed supplemented with the mix was evaluated in terms of its moisture content (total dry extract) by the gravimetric method in an oven at 105 °C for 16 h until it reached a constant weight; lipids were evaluated by Soxhlet extraction; total nitrogen by the Kjeldahl method, multiplying the values of total nitrogen by 6.25 to obtain the equivalent values in protein; ashes in a muffle furnace at 550 °C; carbohydrates by difference, as well as crude fiber [25]. All the analyses were performed in triplicate.

Table 4. Results of the Analysis of the Percent Composition of the Mix of Ingredients

Determinations (%)	Results	% VD*
Moisture	2.41	-
Ashes	3.15	-
Lipids	15.40	28
Carbohydrates	44.22	14.74
Proteins	15.34	20.45
Crude fiber	19.48	77.92
Energy value†	376.84	-

*Daily reference values based on a diet of 2,000 kcal or 8,400 kJ. †kcal/100 g of the product, according to Atwater factors: 9 kcal/g of lipids, 4 kcal/g of protein and 4 kcal/g of carbohydrates.

Statistical analysis

BioEstat version 5.0 software was used for the statistical analysis and the variables are presented as mean and standard deviation (SD). The comparison between the MG and CG was complemented by Student's *t*-test, adopting a 5% level of significance.

Results

The results of the variations in the animals' weight (Table 1) indicated that at the end of the treatment, the weight of the treated group was significantly lower than that of the CG.

Table 2 describes the food consumption of the animals at the beginning and end of the treatment period. Note that the food consumption of two groups did not differ at the beginning of the study, but that by the end of this period the treated group had reduced its consumption significantly in comparison to the CG.

Table 3 shows the results of the biochemical variables. As can be seen, there was a significant reduction in the TC, TG, AST, ALT and glycemia levels and a significant increase in the HDL-c levels.

The results of the analysis of the percent composition show high values of carbohydrates and crude fiber in the formulation used in the experiment (Table 4). Considering that the animals' initial food consumption was 11.71 g, which corresponds to 4.68 g of the mix of ingredients (40:60), these results indicate that the animals' daily consumption of dietetic fiber was ap-

Table 5. Mean and Standard Deviation of the Percentage of Distance Traveled in the Large Intestine of Wistar Rats After Ingestion of the Mixture by the Control Group (CG) and Mix Group (MG)

	CG	MG	P-value*
% traveled	73.38 ± 7.37	97.18 ± 4.61	0.0001

*P-value < 0.05.

proximately 0.92 g.

Table 5 lists the intestinal motility rates. The percentage of distance traveled in the intestines of the MG was greater (15.82 %) than in the CG.

Discussion

The consumption of the formulation used in this study led to a significant decline in weight gain and food consumption of the treated animals (MG). The soy protein, wheat bran and oats in the mix are foods rich in alimentary fibers, which are known to affect body weight. Morenga et al [26] used diets rich in proteins and in fibers for obese patients and observed weight loss in both groups, but this loss was higher in the group on the protein-rich diet.

Other studies have demonstrated the influence of the quality of the diet and of the protein and fiber content on body weight, which is consistent with the results found in this work. The effect of fibers is visible immediately during ingestion, since they increase the mastication time and flow of gastric juices, while the hydration caused by the presence of saliva increases the volume of gastric content, and also hastens and prolongs the body's sensation of satiety, promoting reduced energy intake and a consequent reduction in weight gain [5, 8, 9, 27, 28].

At the end of the treatment, the glucose, TC and TG levels of the MG were significantly lower than those of the CG, and the HDL-c levels were significantly higher, indicating the relevance of the consumption of these components for the prevention and control of risk factors for diabetes and its cardiovascular complications. Weickert et al [29] demonstrated that a diet with high fiber content improved insulin sensitivity by 25% when compared to a protein-rich diet. Fibers also reduce the glycemic index, contributing to the prevention of risk factors for food-related disorders such as diabetes [23, 30, 31].

Studies have shown that the use of linseed (*Linum usitatissimum*) helps lower glycemia and plasma lipids, and increase HDL-c levels [14, 15, 32, 33]. Linseed contains soluble (40%) and insoluble (60%) dietetic fibers that have lipid-lowering activity, including mechanisms that reduce the absorption of cholesterol through the action of viscous gel-forming soluble fibers. Lignins may be responsible for the reducing lipid concentrations. Their metabolites in the organism can bind to bile acids and other chemical compounds such as cholesterol, retarding or diminishing the absorption of these compounds [34-36].

The lipid-lowering effects of linseed can also be attributed to the presence of essential fatty acids ω3 and ω6. Thus, because its omega fatty acids are associated with lignins and vitamins, linseed is considered a functional food with potential antiatherogenic effects [35, 36]. Kaithwas et al [37] demonstrated that *Linum usitatissimum* fixed oil possesses anti-inflammatory, analgesic and antipyretic activities.

The consumption of oats (*Avena sativum*), wheat bran (*Triticum aestivum*) and granola also has positive effects on dyslipidemia in rats and humans. The effects produced by oats are due to the presence of β-glucans, which are resistant to digestive processes, forming viscous solutions in contact with water [38, 39]. Moreover, being pseudoplastic, they slow down digestion, hindering the interaction of pancreatic enzymes with the substrate, thus diminishing the digestion of carbohydrates by pancreatic amylase and reducing the absorption rate of these molecules in the digestive tract. Wheat bran is composed of about 76% of insoluble fibers (cellulose and hemicellulose) and contains several phenolic compounds that act as antioxidants, giving it important properties for the inhibition of LDL-c oxidation and thus reducing the risk for CVD [40, 41].

Visavadiya and Narasimhacharya [42] and Mirmiran et al [43] studied the effects of supplementation with sesame (*Sesamum indicum*) and found lipid and glycemia-lowering effects.

Epidemiological studies have demonstrated the effects of whole grains in reducing the incidence of diabetes type 2, CVD and other chronic disorders, since their compositions contain not only vitamins and minerals but also phenolic compounds, phytoestrogens and other phytochemicals with metabolic properties beneficial in reducing lipid variables, oxidative stress and inflammatory activity [42, 44]. These components are found in sesame, oats, wheat, linseed and granola and can contribute to improve the glycemic profile, as indicated in this study.

Together with fibers, grain-derived proteins seem to contribute considerably to the reduction of TC, as well as other plasma lipids. Soy is considered an important source of protein and several mechanisms of action have been attributed to its bioactive substances, such as flavonoids, and soluble and insoluble fibers, which explain its effect on lipid metabolism [45].

The decrease in cholesterol resulting from the action of soy protein is due to the fact that it is not completely degraded by the digestive enzymes, resulting in the formation of peptides, which can act upon the endogenous cholesterol metabolism and/or may possess properties similar to those of fibers, determining the formation of insoluble complexes with cholesterol that are excreted in stool. Soluble fibers are almost completely fermented in the colon, producing short chain fatty acids that can inhibit hepatic cholesterol synthesis and increase LDLc clearance. Other authors [29, 44, 46] demonstrated that the use of soy protein has a lipid-lowering effect in animals.

Elevated blood levels of transaminases indicate the destruction of liver cells. No such alterations were observed in the present study, indicating that, in the amounts administered to the animals, the mix is safe for consumption [47].

As for intestinal motility, it was found that the distance covered by the activated charcoal from the pylorus to the beginning of the cecum was greater in the rats that consumed the mix (Table 5). This can be attributed to the strong presence of insoluble fibers in the constituents of the mix (Table 4). Dietetic fibers interfere in the weight and consistency of stool, augmenting its motility and diminishing the intestinal transit time, as well as increasing the frequency of bowel evacuation. Chan et al [48] state that a diet rich in fibers is necessary to normalize bowel movements and help form the fecal bolus, claiming that a fiber-rich diet should be part of the treatment of mild constipation. In fact, after evaluating 33 patients with functional constipation, these authors demonstrated that the ingestion of fibers in the diet increased their bowel movements and reduced the need for laxatives. In another study, the subjects received a mixture of breakfast cereals containing 25.0 - 28.7 g of fibers daily for 3 weeks, after which a reduction in the intestinal transit time and a significantly higher frequency of bowel movements were observed [49].

Insoluble fibers have a mechanical effect on the gastrointestinal tract, presenting a low degree of fermentation. By incorporating water (albeit to a lesser extent that soluble fibers), they increase the fecal bolus, facilitate fecal excretion and reduce the intestinal transit time. Since their degradation by bacterial colonies is difficult, they are eliminated almost intact in the feces [50]. The use of the mixture of ingredients in this study promoted a significant increase in the space covered in the large intestine when compared to the control, indicating that it may be helpful in the treatment and prevention of bowel constipation. This problem is associated to one of the most prevalent disorders found by doctors and promotes a negative impact on quality of life.

Conclusions

It was concluded that the mix of soy protein, wheat bran, oats, sesame, linseed and granola, when included in the diet of Wistar rats, had beneficial effects on their body weight and lipid and glycemic profiles, as well as a positive effect in increasing the distance covered in the intestinal transit of these animals. With these results it is possible to say that this mix can prevent or can improve the risk factors of MS, reducing the occurrence of diabetes and CVD. Studies with humans are needed in order to evaluate the occurrence of the same benefits observed in this experimental model.

Conflict of Interests

This work was not supported by any research funding agency. There is no conflict of interest.

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