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Formulation of stevia incorporated cereal-legume based therapeutic food for regulating lipid profile

M.A. Sayed, M.G. Mortuza, M.H. Rashid and M.A. Hossain
Department of Biochemistry, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract: Ground legumes, cereal and stevia (*Stevia rebaudiana* Bertoni) leaves were used in different combination to formulate five kinds of food (F-1, F-2, F-3, F-4 and F-5) to find out effective composition for lowering the serum lipid profile. The experimental rats were fed on the formulated foods: F-1 (74 g cereal + 25 g legume), F-2 (68 g cereal + 30 g legume), F-3 (62 g cereal + 35 g legume), F-4 (56 g cereal + 40 g legume) and F-5 (50 g cereal + 45 g legume). Stevia was added to diet at a rate of 1%, 2%, 3%, 4% and 5% to F-1, F-2, F-3, F-4 and F-5, respectively. Control food was formulated with 20 g glucose, 38.40 g arrow root, 15 g oil and 26.60 g milk powder. The food was supplied at a rate of 15 g/day/rat for ten days and water was supplied *ad libitum*. Food intake was recorded every alternate day. Total food intake was maximum in control group (125.00 g) followed by F-5 (105.27 g), F-4 (105.07 g), F-1 (94.80 g), F-2 (93.38 g) and F-3 group (80.46 g). The total cholesterol levels of rats fed on F-1, F-2 and F-3 were significantly ($P < 0.01$) lower than in control rats. HDL-cholesterol level of rats fed control food (38.29 mg/dl), F-2 (38.32 mg/dl), F-3 (38.17 mg/dl) were statistically similar. HDL-cholesterol level of rats fed on F-4 (26.67 mg/dl) and F-5 (25.07 mg/dl) were also similar. The levels were significantly lower than that of the rats fed on F-1 (45.86 mg/dl). The calculated data on LDL-cholesterol of rats fed on F-1 (4.50 mg/dl), F-2 (10.90 mg/dl), and F-3 (22.02 mg/dl) were lower than that of control rats (43.75 mg/dl). Serum triglyceride (TG) level was the highest in control rats (118.45 mg/dl) compared to those found in F-2, F-3, F-4 and F-5. This study showed a means to reduce the lipid profile of rat through the formulation of cereal and legume based food added with stevia. Thus, it is concluded that therapeutic food formulation can be a tool in the management of cardiovascular disease.

Key words: Formulation, cereal, legume, soybean, therapeutic, lipid profile

Introduction

Cardiovascular disease (CVD) is a global health problem. According to World Health Organization, cardiovascular disease, including coronary heart disease (CHD) is the number one cause of death of an estimated 17.5 million people representing 30 % of the global deaths in 2005. If current trends are allowed to continue, by 2015 an appraised 20 million people will die from these diseases mainly from heart attacks and strokes, which are resulted from high intake of fats.

High plasma cholesterol is positively related to the risk of CHD, which is usually due to an increase in the level of low density lipoprotein (LDL) cholesterol (Carlson and Ericson, 1975; Gordon *et al.,* 1977; Grundy, 1987). Elevated LDL cholesterol and reduced high density lipoprotein (HDL) cholesterol in the plasma have been independently associated with increased risk for CHD (Gordon *et al.,* 1977). High level of plasma triglycerides is also considered a risk factor for CHD (Ahmed and Basser, 1993).

Many food legumes are regularly consumed by human and animals as an important source of protein for their nutrition. Legume consumption is significantly and inversely associated with the risk of CHD and CVD after adjustment for established CVD risk factors. Soy-based foods are an important source of dietary protein in Asian countries and its supplementation to the diet reduces serum cholesterol (Bazzano *et al.,* 2001; Potter *et al.,* 1998). A study with Chinese women found that soy food consumption was associated with a lower risk of coronary disease (Zhang *et al.,* 2003). Interestingly, diets low in fat and high in carbohydrates from grains, fruits and vegetables are associated with a lower risk of cardiovascular disease (Lichtenstein *et al.,* 1998).

Stevia (*Stevia rebaudiana* Bertoni) is an exotic plant in Bangladesh. It has both economical and medicinal importance, and has significant effect on lowering the serum lipid profile (Savita *et al*., 2004). With the ever increasing incidence of cardiovascular diseases, its management has become costly not only for the individual but also for the nation as a whole. The globe witnesses an explosive increase in cardiovascular diseases, demanding intensified prevention and treatment not least for the low income population. Dietary trials have clearly established that the combination of different diet or supplementation of cereal based foods with bean/legumes lower the serum cholesterol level. Based on the above consideration, this study was carried out on rats to elucidiate the effect of formulated foods using legumes, cereal and stevia in various combination to find out the appropriate composition effective for lowering the lipid profiles with a view to manage the high incidence of CVD.

Materials and Methods

The study was conducted in the Department of Biochemistry, Bangladesh Agricultural University, Mymensingh, Bangladesh in May, 2009. Sun-dried seeds of wheat (*Triticum aestivum*), lentil *(Lens culinaris* Medik), chickpea (*Cicer arietinum* L), black gram (*Vigna mungo*), grasspea (*Lathyrus sativus* L.) mungbean (*Vigna radiata* L.), and soybean (*Glycine max*) were collected from local market of Mymensingh. Seeds were dehulled, sundried and ground by grinder. Stevia (*Stevia rebaudiana* Bertoni) leaves were collected from Banglasdesh Sugarcane Research Institute (BSRI), Pabna, Bangladesh. Dried leaves were ground by mortar and pestle and preserved in air tight containers.

Food formulation: All the ingredients along with vitamin premix were added as per formula of experimental diets and mixed homogenously (Table 1 and 2). Required amount of water was added to make the mixture moist to be made into dough. The resultant dough was then passed through a 1 mm diameter pelleting machine. Finally pellets were sun dried for two days and separately stored in airtight plastic boxes.

Experimental animal: Eighteen mixed albino rats of male sex (Long Evan's strain) used in this experiment were procured from International Center of Diarrheal Disease Research, Bangladesh (ICDDR, B).

Experimental procedure: Thirty –day-old male Long Evans rats were randomly divided into six groups (each group of three) and housed individually in Perspex cages.

After 6 days of adaptation, animals were segregated on the basis of their body weight; rats were fed for 7days on standard rat chow (15 g/day/rat) before being put on experimental diets for 10 days. Distilled water was made available *ad libitum.*

Acclimatization of rats and experimental design: The experiment was laid out in Complete Randomized Design (CRD) with three replications. After acclimatization, the rats were ready for experiment. Six groups of rat's viz. control, F-1, F-2, F-3, F-4 and F-5 were fed for 10 days. Each rat was fed with formulated 15 g food/day.

Observation of Rats: All the rats were examined twice daily for any visible physical changes like restlessness, lordosis, abnormal gait, vices and depression as well as feeding style during treatment.

Collection of blood serum and estimation of lipid profile: At the end of experiment, all rats were exposed to mild ether anesthesia. Then blood was collected with a syringe and needle directly through cardiac puncture without using any anticoagulant. After centrifugation of collected blood, the supernatant were carefully collected by a micropipette and preserved in eppendorf vial. The collected serum was stored at 15^0 C. These serum samples were used to determine total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides levels.

Statistical Analysis: The results were subjected to analysis of variance (ANOVA) by using the MSTATC programme. When *P* values were <0.01, the significance of difference between groups were estimated by Student's-t-test.

Results and Discussion

Effects of formulated foods on food intake of rats: Data are presented on daily basis to observe the trend of food intake. The rate of food intake by the different rats varied from day to day throughout the experimental period (Table 3). The total food intake was maximum in rats fed on control food (125.00 g) followed by F-5 (105.27 g), F-4 (105.07 g), F-1 (94.80 g), F-2 (93.38 g) and F-3 fed group (80.46g). This variation in food intake might be due to different palatability of foods. Food intake trend found in this study differed from that reported by Imaizumi *et al.* (1990). They used raw palm oil and lard in their experiment and observed no significant differences in food intake. However, in this experiment variations in palatability may be due to the different ingredient of food. Control food contains the entire ingredient that is responsible for the best palatability of the food.

Table1. Formulations of control food (100 g weight basis)

Ingredient	Amount (g)
Glucose	20.00
Arrow-root	32.40
Oil	15.00
Milk powder	32.60
Vitamin premix	0.25

Table 2. Formulation of test foods (100 g weight basis)

Ingredients	Food 1 (g)	Food 2 (g)	Food 3 (g)	Food 4 (g)	Food 5 (g)
Wheat	74	68	62	56	50
*Legumes	20	20	20	20	20
Soybeans	5	10	15	20	25
Stevia	1	2	3	4	5
Vitamin premix	0.25	0.25	0.25	0.25	0.25

*Legumes included lentil, chickpea, grasspea, mungbean and blackgram at the rate of 4 g in each food i.e. total 20 g per 100 g weight basis.

Table 3. Intake of the experimental diets

Group	Food intake (g/day)					Total food Intake (g)
	2^{nd} days	4^{th} days	6^{th} days	8^{th} days	10^{th} days	
Control	28.01	27.01	26.01	20.33	23.67	125.00
F-1	12.92	20.54	18.67	26.03	16.67	94.80
F-2	19.41	15.97	13.33	24.02	20.67	93.38
F-3	9.43	21.03	18.02	18.03	14.02	80.46
F-4	13.07	26.02	19.33	28.67	14.05	105.07
F-5	10.47	25.80	19.33	25.67	24.03	105.27

Values are expressed in (Mean)

Effects of formulated foods on serum lipid profile of rats: The level of serum lipid profile of rats fed on different foods varied significantly (Table 4). The values of total cholesterol level in rats of group F-1, F-2, F-3, F-4 and F-5 were 62.27, 60.87, 60.13, 68.80 and 78.40 mg/dl, respectively. Among the treatment groups, the level in group F-1, F-2, and F-3 was significantly higher than that in group F-4 and F-5. However, expectedly this parameter in all groups was significantly lower than in the control rats. This finding is in agreement with the previous reports (Furga and Dierzuk, 2006; Park and Lee, 2003 and Gorecka *et al.*, 2003) who reported that cereal and legume added to diets for rats lower total serum cholesterol.

Among the treatment groups the values of HDL-cholesterol level in rats of group F-2 (38.32 mg/dl), F-3 (38.17 mg/dl) and control (38.29 mg/dl) was significantly ($P<0.01$) higher than that in group F-4 (26.67 mg/dl) and F-5 (25.07 mg/dl). However, expectedly this parameter in all groups was significantly lower than in the F-1 rats. This finding is in agreement with the result reported by Macarulla *et al*. (2001). They concluded that legume (*Vicia faba*) added to diets for rats increases HDL-cholesterol.

The calculated data on LDL-cholesterol exhibited that LDL-cholesterol level was higher in control food group (43.75 mg/dl) while it was lower in F-1 (4.50 mg/dl) group. The values obtained in F-1 (4.50 mg/dl), F-2 (10.91 mg/dl), F-3 (22.02 mg/dl) and F-4 (40.84 mg/dl) were significantly different from that of control group (43.75 mg/dl). This finding is in agreement with the result reported by Macarulla *et al*. (2001) and Savita *et al*. (2004) who observed that legume and stevia added to diets for rats lower LDL-cholesterol.

The values of Triglyceride level in rats of group F-1, F-2, F-3, F-4, F-5 and control were 59.50, 58.23, 59.74, 61.27, 62.44 and 118.45 mg/dl, respectively. Among the treatment groups, the level in rats of group F-1, F-2, F-3, F-4 and F-5 were significantly lower than that in control rats. This finding is in agreement with the results reported by Macarulla *et al*. (2001) and Savita *et al*. (2004). According to them legume and stevia added to diets for rats lower triglyceride.

Table 4. Effects of formulated foods on several blood parameters of rats fed on the experimental diets for ten days

Blood Parameter	Groups						Level of significance
	Control (mg/dl)	F-1 (mg/dl)	F-2 (mg/dl)	F-3 (mg/dl)	F-4 (mg/dl)	F-5 (mg/dl)	
Total Cholesterol	105.47a	62.27d	60.87d	60.13d	68.80c	78.40b	**
HDL-Cholesterol	38.29b	45.86a	38.32b	38.17b	26.67c	25.07c	**
LDL-Cholesterol	43.75 a	4.50e	10.90d	22.02c	30.17b	40.84a	**
Triglyceride	118.45a	59.50b	58.23b	59.74b	61.27b	62.44b	**

Values are expressed in mg/dl (Mean); ** = Significant at 1% level of probability; in a column with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT).

Formulated therapeutic foods have been found to decrease lipid profile by lowering the total cholesterol, LDL-cholesterol and Triglyceride and to increase the HDL-cholesterol in albino rats. Food-3 was the most effective among the formulated foods. Future study to identify the ingredient(s) present in the formulated food responsible for lowering the lipid profiles except HDL-cholesterol will be stressed.

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