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Development of a modified hypercholesterolaemic rat model to study the efficacy of hypocholesterolaemic therapeutic agents

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Abstract
Raised cholesterol increases the risk of heart disease and stroke. The management of hypercholesterolaemia by administration of hypocholesterolaemic agents, either in the form of novel pharmaceuticals or herbs, is a major research area at present. A hypercholesterolaemic animal model is a prime requirement to test the efficacy of the testing agent. According to the literature the cost is very high for the establishment of hypercholesterolaemic rat model using commercial cholesterol. The present work aimed at establishing a dietetic rat model capable of promoting a moderate level hypercholesterolaemia to be used in the area of lipid research.

Hypercholesterolemia was induced by feeding a diet containing 10% butter incorporated into standard rat and mice feed formula for a period of twelve weeks. Fasting serum cholesterol levels were measured at four, eight and twelve week intervals.

The fasting serum cholesterol concentration of rats fed on hypercholesterolaemic diet was found to be significantly higher (p<0.05) compared with the control group.

Present study confirms the development of a diet based, cost-effective and time efficient experimental rat model, to explore the efficacy of novel therapeutics in the area of lipid research.

Keywords: Hypercholesterolaemia, dietetic model, hypercholesterolaemic rat model, butter induced hypercholesterolaemia

1. Introduction

Hypercholesterolaemia is a common metabolic derangement debilitating population in both affluent and less-affluent societies alike [1, 2]. Raised cholesterol increases the risk of heart disease and stroke [3, 4]. According to WHO global health observatory data, raised cholesterol accounts for one third of ischemic heart diseases worldwide [5]. It is a concern for health professionals, since the high cholesterol levels contribute as a risk factor for the development of cardiovascular diseases, such as atherosclerosis and its complications [6].

Management of hypercholesterolaemia by administration of hypocholesterolaemic agents is a demanding field of research and continuously novel products are being tested. To study the therapeutic value of different hypocholesterolaemic agents (synthetic or herbal) it is essential to maintain an easy handling hypercholesterolaemic animal model. A scanty literature was available to gather information on hypercholesterolaemic animal models despite its greater importance in this field of research.

A large number of animal models, such as pigeons, chickens, swine, cats, dogs, non –human primates; mice, rabbits and rats have been tested by several workers during the recent past [7-11]. Male Wistar rats are a convenient and inexpensive experimental animal model, due to their small size and lower intake of food [8].

In order to induce hypercholesterolaemia in rats, triglycerides-rich diets containing cholesterol, with or without cholic acid have been used by Lichtman *et al*., [12]. It has been shown that rats could be made hypercholesterolaemic by feeding diets enriched with 1 - 2% of cholesterol, fat, bile salts and sucrose [12]. Another study revealed that a feed containing 0.25% and 0.5% cholesterol (standard for chromatography) could be used to induce hypercholesterolaemia in experimental rats [7]. The authors succeeded in increasing the blood cholesterol levels in rats by 68 ±19% and 80±14% respectively in two experimental diets (0.25% cholesterol and 0.5% cholesterol) with commercial cholesterol. Due to high cost of pure cholesterol, an attempt was made by Thammitiyagodage *et al*., (1999) to achieve a comparable increase in blood cholesterol by suitably modifying the experimental diet used by Samaranayake *et al.*, (1998) [8, 7]. This experimental diet contained 0.25% cholesterol (standard for chromatography) and butter (5%). Though it was a successful hypercholesterolaemic rat model, due to the high cost of commercial cholesterol the experimental feed was not cost effective.

Therefore, in the present study an attempt was made to replace the high cost commercial cholesterol from the experimental diet and to substitute with low cost butter (Highland) to establish a hypercholesterolaemic rat model to be used to test the efficacy of hypocholesterolaemic therapeutic agents.

2. Martials and Methods

2.1. Chemicals and equipment

Ingredients to prepare the rat feed including butter (Highland) were purchased from the local market in Colombo, Sri Lanka. Assay kits for blood cholesterol were purchased from Sigma – Aldrich (P.O. box 14508, St, Louis, MO 63178, USA).
Shimadzu UV 1601 visible spectrophotometer (Simadzu Corporation, Kyoto, Japan) was used to measure the absorbance. Feed pellets were dried by using the pellet drying machine (Takashma Shoten Co. Ltd) with a control temperature at 60 °C ±5 °C.

2.2. Experimental animals and their management

After obtaining the permission (protocol approval No. 538/11) from the Ethics Review Committee of the Faculty of Medical Sciences (approved Ethical Review Committee of SIDCER, Strategic Initiative for Developing Capacity in Ethical Review), University of Sri Jayewardenepura, Sri Lanka, experiments were conducted on male Wistar rats (obtained from Medical Research Institute, Sri Lanka) weighing approximately 250-280 g. The animals were housed for one week, in a 12:12 hours light and dark cycle in temperature and humidity-controlled room. The animals were given free access to food and water. After one-week adaptation period, the animals were used for the study.

2.3. High fat diet

High fat diet was prepared by incorporating 10% butter (Highland) into WHO standard feeding formula which was obtained from the Medical Research Institute (MRI), Sri Lanka [13]. Ingredients of each feed (test and control) are shown in Table 1.

Table 1: Composition of the diets (g/1000g of diet) fed to control and test groups of animals.

Ingredients	Quantity
Maize	405
Brown rice	100
Rice bran	25
Wheat bran	20
Wheat flour	135
Fish meal	80
Soya meal	80
Milk powder	60
Soya oil	20
Grass powder	30
Bone meal	15
Vitamin mix	2.4
NaCl	2.0
Beta mix E 50	0.2
DL methionine	0.5
B complex	6
Molasses	25

*The test feed was prepared by incorporating 100g of butter into control feed (1000g)

2.4. Induction of hypercholesterolaemia in experimental animals

The animals were randomly divided into two groups (n=6 in each group) and the control group was fed a standard diet. Hypercholesterolaemia was induced in the test group by feeding high fat diet over the experimental period. Body weights were recorded weekly and a general observation was made on the daily food intake.

2.5. Blood biochemical analysis

At the end of four, eight and twelve weeks of feeding the control and tests, the animals were fasted (12-14 hours) and blood (0.5 ml) was collected by tail-vein puncture from the light ether–anesthetized rats and subjected to centrifugation to obtain serum. Total serum cholesterol was determined by the enzymatic method using diagnostic kits obtained from Sigma – Aldrich (USA).

2.6. Composition and energy values of control and high fat feed

Percentage composition of fat, protein, carbohydrates, crude fiber, ash and moisture was determined following methodology proposed by Association of Analytical Chemists, Official Methods of Analysis (AOAC) [14].

2.7. Statistical analysis

All the results of the experiments were expressed as mean± standard deviation (Mean±SD). The measurements were performed in duplicates. Data pairs were compared by using the Student's T-test in Microsoft Excel. $p<0.05$ were considered to be statistically significant.

3. Results and Discussion

Various models are found in the literature for the study of hypercholesterolemia in rats. A much higher increase (80%) in the serum cholesterol level has been reported by Samaranayake *et al*., (1998) [7]. This feed formula contained 0.5% cholesterol standard for chromatography. In another feeding trial an increase in random cholesterol (78.4%) and that of fasting serum cholesterol (94.5%) was observed by Thammitiyagodage *et al*., (1999) and the feeding formula contained 5% butter and 0.25% cholesterol as the inducer of hypercholesterolaemia [8]. A feeding trial which was conducted using soya bean and cholesterol succeeded in establishing a hypercholesteroalemic rat model [11]. However, the cost of commercial cholesterol to provoke hypercholesterolaemia is very high and therefore funding needs to purchase cholesterol is a major drawback in this area of research though it's a prerequisite.
Considering the cost of the pure cholesterol, the present study was designed to establish a cost effective, easy handling laboratory model which could be used by many scientists in testing potential therapeutic value of different hypocholesterolaemic agents.
In the present study, eight weeks after the dietetic treatment, the fasting serum cholesterol level (Table 2) of the test group (fed on diet containing butter) was significantly higher when compared with the control group ($p < 0.05$). After twelve weeks of the feeding trial the test group presented a significant but lesser increase in cholesterol level when compared to those values obtained for eight weeks. The increase in fasting cholesterol thus was 34% and 30% in eight weeks and twelve weeks respectively compared to control animals.
According to the evidence shown in Table 2**, p**ercentage increase in the body weight of control rats at 8 and 12 weeks were 22% and 24% respectively when compared with their base line values. Test group showed a much higher increase in the weight gain at the corresponding time intervals and that

was 24% and 27% respectively in comparison with their base line values.

The remarkable feature of this experimental diet is that it was prepared by using ingredients (Table 1) of significantly less cost when compared with previously established models. Since the hypercholesterolaemic feed was prepared by using 10% commercial butter (Highland) incorporated with standard rat and mouse feed used in the Medical Research Institute, Sri Lanka, we were able to reduce the cost of the diet permitting this model to be used in the area of lipid research.

Another important observation made during the experimental period was higher but non-significant weight gain of the test animals when compared with the controls despite their less food intake. It was further noted that the food intake of the test group was comparably less. This was especially interesting and possibly the high fat content of the test feed which had a high caloric value led to its lower ingestion by test group animals (Table 3). It is suggested that consequently there would be a lower ingestion of other nutrients too and that would induce malnutrition in test animals [15]. According to the available scientific evidence the rat, to a certain extent, ingest a diet driven by its caloric content and possibly that could be the reason attributing to less food intake by the rats of the test group [15].

Table 2: Body weight and cholesterol levels of rats fed on control and high-fat diets for four weeks, eight weeks and twelve weeks.

	Body weight (g)		**Cholesterol level (mg/dL)**	
	Control	Test	Control	Test
Baseline value	279.1±10.1	275.0±8.8	84.0 ±8.7	92.9±7.8
After 4 weeks	323.3±17.5	304.1±12.8	92.9±4.1	92.4±2.98
After 8 weeks	341.6±26.2	320.0±16.7	95.7±23.0*	128.3±5.5*
After 12 weeks	347.3±33.2	328.3±21.5	103.1±.7.6*	133.0±6.7*

*p<0.05 when compared with control, values are represented as mean± SEM (n=6).

Table 3: Percentage composition and energy value of control and test feed

Feed variety	**Percentage Composition**						**Energy Value kcal/kg**
	Fat	Protein	Carbohydrate	Crude fiber	Ash	Moisture	
Test	15.7	15.4	57.5	2.3	5.4	3.7	4329
Control	5.8	15.0	68.3	2.1	5.3	3.5	3855

4. Conclusion

The present study provides evidence on establishing a cost effective easy handling hypercholesterolaemic rat model to be used in testing for hypocholesterolaemic efficacy of different therapeutics.

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