EFFECTS OF BUTTER ON BLOOD BIOCHEMISTRY AND HISTOTEXURE OF HEART IN THE DEVELOPMENT OF OBESITY IN SWISS ALBINO MICE

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ABSTRACT

The study was designed and performed in order to investigate the effects of butter on blood biochemistry and histo-texture of heart in mice. Thirty days old 30 Swiss Albino mice were divided into 3 equal groups (M, M_1, and M_2). Mice of group M were fed with normal mice pellet and were designated as control. Mice in the group M_1 and M_2 were fed with 10% and 20% butter supplemented feed respectively. In biochemical study, group M_2 showed increase in total plasma cholesterol ($P<0.001$), LDL ($P<0.001$), TG ($P<0.001$), blood glucose ($P<0.05$), blood urea nitrogen ($P<0.05$) compared to mice in control group. In group of M_1 there were also increase in total plasma cholesterol ($P<0.01$), LDL ($P<0.001$), TG ($P<0.001$), blood glucose ($P<0.05$), blood urea nitrogen ($P<0.05$) compared to mice in control group but lesser increased than the mice fed with 20% butter supplemented feed. In histopathological study there was no significant change in the heart of mice.

Key words: Mice, Obesity, Butter, Hypercholesterolemia, Hypocholesterolemia

INTRODUCTION

Obesity is a medical condition in which excess body fat is accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. A person is obese when Body Mass Index (BMI) is greater than $30\,kg/m^2$ (WHO, 2000). Obesity is a major health problem worldwide. In United States, roughly 300,000 deaths per year are related to obesity. Obesity also increases the risk of developing several chronic diseases such as type 2 diabetes, insulin resistance, coronary heart disease (responsible for heart attacks), cerebrovascular disease (responsible for strokes), high blood pressure, gout, gallstones, colon cancer, sleep apnea, and a form of liver disease called nonalcoholic fatty liver disease (NAFLD) (Haslam and James, 2005). Clinical and epidemiological studies have led to the identification of the genetic, environmental, and inflammatory risk factors of these diseases like dyslipidemia, hypertension, diabetes, obesity, smoking, high-fat diet, fatty liver disease, and chronic inflammation (Glass and Witztum, 2001). According to the National Health health care systems and Nutrition Examination Survey (NHANES), between 1999 and 2010 the overall age-adjusted prevalence of obesity in the USA was 35.8% in adult men and 35.5% in adult women (Flegal *et al.*, 2010). It is now well established that obesity is an independent risk factor for the development of cardiovascular diseases. Cardiovascular disease (CVD) is a degenerative disease that most often occurs and becomes a major killer in industrialized countries. In developing countries, the incidence of cardiovascular disease is increasing alarmingly (Okrainec *et al.*, 2004). CVD is emerging as a serious health hazard in Bangladesh. Latest survey on cardiovascular diseases in Bangladesh showed prevalence of ischemic or coronary heart disease (CHD) in adult population about 10% (National Heart Foundation of Bangladesh, 2010).

A major cardiovascular disease of the productive age is coronary heart disease (CHD), which is closely related to atherosclerosis (Kalim *et al.*, 1996). Compared to men, women suffer a disproportionate burden of disease attributable to overweight and obesity. Female obesity is defined as an excessive amount of body fat, keeping in mind the Body Mass Index ratio, of the woman concerned. The body mass index (BMI), is the most popular and effective way of calculating female Obesity. The occurrence of CVD can be reduced by decreasing the formation of atherosclerosis through lowering cholesterol levels in the blood and increasing the concentration of high density lipoprotein (Nogrady, 1992). Generally, high-fat diets, high sucrose/fructose diets, diets high in saturated fats and restricted in certain essential nutrients, like choline and methionine, have been shown to cause obesity and fatty

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livers in a number of different strains and species of rodents (Koteish, 2011). High-fat/high-cholesterol western diets induce extreme hypercholesterolemia and also lead to concomitant features of the metabolic syndrome, such as weight gain, decreased HDL levels, obesity, hypertriglyceridemia, hyperinsulinemia, and insulin resistance (Hartvigsen *et al*., 2007; Li *et al*., 2000). In addition, these diets generate pathologies independent of atherosclerosis, such as changes in fur and skin integrity, changes in plasma lipids, and hepatic steatosis (Lichtman *et al*., 1999; Gajda *et al*., 2007). This suggests that "overnutrition" might play a role in the genesis of obesity related fatty liver disease and other risk factors associated with metabolic syndrome (Gajda *et al.*, 2007). Dietary cholesterol has been shown to reduce fatty acid oxidation, which in turn increases the levels of hepatic and plasma triacylglycerols (TG) (Fungwe *et al*., 1993). Evidences from lipid lowering trials have clearly established that reduction of total cholesterol or low density lipoprotein cholesterol (LDL-C) is associated with decreased risk of atherosclerosis and CHD (Brown *et al.*, 1998; Grundy *et al.*, 2005). The ingestion of polyunsaturated fatty acids present in vegetable oils is inversely related to the incidence of heart diseases by decreasing the cholesterol and triacylglycerol plasmatic levels. Increased adiposity and insulin resistance contribute to the progression from NASH to fibrosis through the development of a profibrotic milieu in the liver, including increased hepatocellular death (Chiang *et al*., 2011). Commercial butter is about 80% butter fat and 15% water; traditionally made butter may have as little as 65% fat and 30% water containing high proportion of saturated fat. Saturated fat consists of triglycerides containing only saturated fatty acids having no double bonds between the individual carbon atoms of the fatty acid chain. That is, the chain of carbon atoms is fully "saturated" with hydrogen atoms (Jakobsen *et al.*, 2009). Chemically butterfat consists essentially of a mixture of triglycerides, particularly those derived from fatty acids, such as palmitic, oleic, myristic, and stearic acids. Fats rich in saturated fatty acids can result in the elevation of plasma total and lipoprotein cholesterol (Yuan *et al.*, 1998). Changes in the diet in terms of fatty acid content can have a marked effect on lipid profile of blood. Therefore, the investigation was made by the application of high fat to learn about any alteration in biochemical blood profile of and in histo-texture of heart in mice.

MATERIALS AND METHODS

The experiment was conducted in the Department of Physiology, Bangladesh Agricultural University, Mymensingh during a period of 3 months from 25 September through 25 December 2013.

Animals

One month old 30 Swiss Albino mice with an average body weight of 15-18 gm were purchased from ICDDR, Bangladesh, Mohakhali, Dhaka. Before being used in the experiment, mice were adapted for 7 days in order to acclimatize in the environment. All groups were housed in a compartmentalized rectangular metallic cages ($9 \times 11 \times 7$ cubic inches) wrapped with wire mesh. The cages were kept in well ventilated room at 28 ± 2^0C and a relative humidity of 70-80% with natural day and light.

Experimental design

The mice were randomly divided into 3 equal groups (M, M_1 and M_2). Each group consisted of 10 mice (n=10). Group M was kept as control and animals were fed with normal broiler pellet (HI-PRO-VITE feed; 5gm/mice/day). Mice of group M_1 and M_1 were fed with 10% and 20% butter with feed, respectively. Butter was collected from local KR market of Bangladesh Agricultural University (BAU), Mymensingh. All groups were supplied with standard broiler pellet (HI-PRO-VITE feed; 5gm/mice/day) and fresh drinking water was given ad libitum throughout the experimental period of 90 days.

Collection of samples

On the 1st day (after acclimation) from the tail and at the end of the experiment, blood sample was collected by sacrificing the mice. Sera samples were separated and stored at -20^{0}C temperature until being used. On the 90th day of the experiment, heart from the experimental mice were collected by sacrificing in 10% buffered formalin and used for histopathological study.

Biochemical studies

The biochemical parameters of serum like Total Cholesterol, Triglyceride, HDL, LDL, Blood glucose, Billurubin, Blood urea nitrogen, serum creatinine, ALT, AST, were estimated.

Determination of total serum cholesterol and triglycerides

The cholesterol was determined using the procedure described by Trinder (1969). The result was expressed in mg/dl.

The triglyceride of blood serum was determined by Biochemistry Humalyzer-3000 (Human type, Germany) according to the technique described by Trinder (1969). The result was expressed in mg/dl.

Determination of HDL and LDL cholesterol

The concentration of serum HDL cholesterol was estimated with the incubation of supernatant of serum sample and reagent mixture in Reflectron® Humalyzer 3000 (Human type, Germany) and then placing the mixture in the Reflection® against the blank reagent. The result was expressed in mg/dl. The LDL was determined by subtracting the HDL cholesterol value from the subtracted value of triglyceride from total serum cholesterol that was divided by five.

$$\text{LDL LDL-C} = \frac{\text{Total serum Cholesterol} - \text{Triglycerides}}{5} - \text{HDL-C}$$

Determination of blood glucose

The Blood glucose was determined after enzymatic hydrolysis with amylases. The blood glucose was determined by Biochemistry Humalyzer-3000 (Human type, Germany) according to GOD TAT method. The result was expressed in mg/dl.

Determination of AST & ALT

AST and ALT values were recorded through placing the mixture of serum sample and test reagent (enzyme/coenzyme/α – oxoglutarate AL 1205) in the Humalyzer 2000. The AST value was calculated as: AST/ALT Concen. = $1746 \times$ absorbance U/L.

Determination of Bilirubin and Blood Urea Nitrogen

Bilirubin estimation was performed according to the method described by Peterson *et al.* (1952). The result was expressed in mg/dl. Blood urea nitrogen was determined applying the Modified Berthelot Methodology and expressed as gm/dl.

Histopathological study

Histopathological examination was performed in the Department of Anatomy and Histology, BAU, Mymensingh. Fixed tissue sections were processed; paraffin-embedded and sectioned were routinely stained with Hematoxylin and Eosin (H & E) stain as per standard procedure.

Statistical analyses

All data were expressed as mean ± SD, and differences among the groups of animals were compared using one-way ANOVA with post-hoc Duncans test. Paired t- tests were used to compare pre-treatment and post-treatment value of different groups. Statistical significance was set at $P < 0.05$. Statistical analysis was performed using SPSS software version 17 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

In the present study the result shows that effects of various percentages of butter on blood biochemistry vary significantly. In case of blood sugar both percentages (10% and 20%) of butter supplemented feed signifies the result at ($P<0.05$) compared to control group that is in agreement with the study of Hoefel *et al.* (2011) who reported that in liver, glycogen synthesis and concentration were higher in rats receiving hypercaloric saturated fat (HSF) and normocaloric saturated fat (NSF). The significant result of TG ($P<0.001$) in the present study could be compared with the study of Bałasińska and Mazur (2004) who reported that dietary lipids participate actively in the development of atherosclerosis which is characterized by elevated triglyceride levels in blood plasma. Again the significant result of TC ($P<0.001$) and LDL ($P<0.01$) in this study is similar with the study of Ohlsson (2010) who reported that the saturated fatty acids increase total plasma cholesterol, especially LDL-C. In case of

blood urea nitrogen and serum creatinine result is significant at (P<0.05) level that could be compared with the study of Jing Lu *et al*. (2003) who showed that high fat diet causes detrimental effect on kidney, causing renal inflammation and fibrosis resulting low clearance of creatinine and increase in blood urea nitrogen. In the histopathological section of heart, there was no significant changes among control group, 10% butter treated and, (c) 20% butter treated groups (Fig. A, B and C). The present research findings help in drawing a conclusion that 20% butter supplementation would be able to cause changes in biochemical parameters of blood.

Table 1. Effects of various percentages of butter on blood sugar, bilirubin, TC, TG, HDL and LDL, in Swiss albino mice

Parameters	Groups	Pre treatment	Post Treatment	Level of Significance
Blood Sugar (mg/dl)	M (Control)	45.00±1.35	48.26±1.05[b]	NS
	M$_1$ (10% butter)	44.00±1.43	95.13±1.64[a]	*
	M$_2$ (20% butter)	43.50±1.90	102.92±1.14[a]	*
Bilirubin (mg/dl)	M (Control)	0.20±0.01	0.19±0.02[b]	NS
	M$_1$ (10% butter)	0.19±0.02	0.20±0.02[b]	NS
	M$_2$ (20% butter)	0.18±0.02	0.19±0.06[b]	NS
TC (mg/dl)	M (Control)	45.00±1.10	48.74±1.20[c]	NS
	M$_1$ (10% butter)	44.23±1.01	84.52±1.38[a]	**
	M$_2$ (20% butter)	44.90±1.00	132.38±1.89[a]	***
TG (mg/dl)	M (Control)	46.32±1.00	53.60±0.75[c]	NS
	M$_1$ (10% butter)	45.56±0.70	103.70±0.65[a]	***
	M$_2$ (20% butter)	45.43±0.71	139.04±1.56[a]	***
HDL (mg/dl)	M (Control)	18.50±1.09	20.05±1.04[b]	NS
	M$_1$ (10% butter)	18.00±1.10	19.10±1.14[b]	NS
	M$_2$ (20% butter)	17.87±1.08	19.00±1.23[c]	NS
LDL (mg/dl)	M (Control)	26.23±1.30	29.37±1.74[c]	NS
	M$_1$ (10% butter)	25.00±2.15	95.71±1.78[a]	***
	M$_2$ (20% butter)	25.58±2.50	97.42±3.43[a]	***

Table 2. Effects of various percentages of butter on AST, ALT, and BUN in Swiss albino mice

Parameters	Groups	Pre treatment	Post Treatment	Level of Significance
AST (U/L)	M (Control)	40.12±0.23	41.82±2.48^c	NS
	M$_1$ (10% butter)	41.23±0.43	41.90±2.08	NS
	M$_2$ (20% butter)	41.56±0.53	42.67±3.06^b	NS
ALT (U/L)	M (Control)	22.35±1.78	22.67±1.55^c	NS
	M$_1$ (10% butter)	23.21±1.10	24.00±2.00^b	NS
	M$_2$ (20% butter)	22.48±1.03	23.00±2.00^b	NS
BUN (gm/dl)	M (Control)	25.00±1.35	25.46±1.18^b	NS
	M$_1$ (10% butter)	25.01±1.45	31.97±1.63^b	*
	M$_2$ (20% butter)	26.07±1.35	32.92±0.74^b	*

The present study is a preliminary work on laboratory animal in this area. The study is in limitation due to the period of time, percentage of butter supplementation, environmental facilities, no. of animals used and so on. Hence there remains an intension to perform the study in detail in future with the overcome of the present impediments that would be able to get a rigid and confirm idea about obesity and its related complications in mammals and that could be used as a model for human.
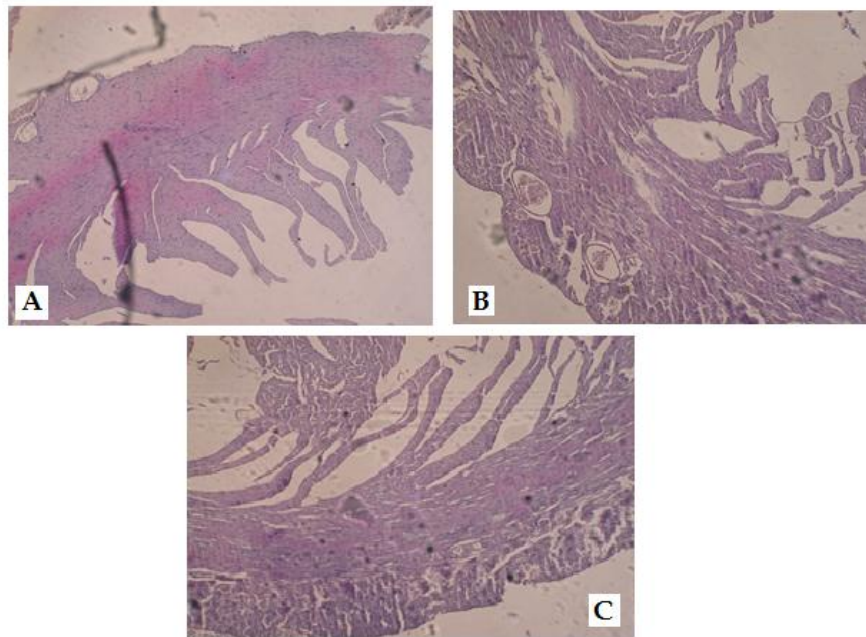

Fig. 1. Histopathological lesions: A. Control group, B. 10% Butter treated group, and C. 20% butter treated group.

M. S. Islam and others

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