EFFECTS OF BUTTER AND ESTROGEN ON LIPID PROFILE AND HISTOTEXURE OF LIVER AND SKIN IN REFERENCE TO THE DEVELOPMENT OF OBESITY IN SWISS ALBINO MICE

MS Islam[1], MK Islam[2], and SK Das[3]

[1]School of Agriculture and Rural Development, Bangladesh Open University, Gazipur 1705, Bangladesh

[2]Department of Physiology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

[3]Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

ABSTRACT: The study was performed in to investigate the effects of butter and estrogen on lipid profile and histo-texture of liver and skin in mice. 30 days old 60 (30 male and 30 female) Swiss Albino mice were divided into 6 equal groups of which (M, M_1, and M_2) were in male and (F, F_1, F_2) in female. Group M and F (Control) were fed with normal mice pellet. Mice in the group M_1 and F_1 (butter treated) were fed with 20% butter; group M_2 and F_2 (estrogen) were fed with estrogen @ of 10 µg/mice/day. The effect of butter and estrogen in the development of obesity in mice was evaluated based on weight gain, lipid profile, blood glucose and histopathology. In this study in case of male the highest body weight gain was detected in group M_1 ($P<0.001$) and in biochemical study, group M_1 showed the increase in total plasma cholesterol ($P<0.01$), LDL ($P<0.01$), TG ($P<0.001$), blood glucose ($P<0.05$) but increase in HDL ($P<0.001$) in M_2 group compared to control group. In female the highest body weight gain was detected in group F_1 ($p<0.001$) and in biochemical study, group F_1 showed the increase in total plasma cholesterol ($P<0.01$), LDL ($P<0.01$), TG ($P<0.001$), blood glucose ($P<0.05$) but increase in HDL ($P<0.001$) in F_2 group in compared to control group. In histopathological study fatty changes and endothelial desquamation were found in the liver of butter treated groups. Fatty changes, loss of elasticity, dermal thickness were found in the skin of butter treated mice. On the basis of the findings and observations of the present study, it can be concluded that 20% butter supplementation would be able to cause a change in lipid profile and would also produce degenerative changes in liver and skin of mice.

KEYWORDS: Obesity, Cholesterol, lipid, Estrogen, Cholesterolemia.

1 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 it is greater than 30 kg/m^2. 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). 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 [1].

Obesity is most commonly caused by a combination of excessive food energy intake, lack of physical activity, and genetic susceptibility, although a few cases are caused primarily by genes, endocrine disorders, medications or psychiatric illness. Evidence to support the view that some obese people eat little yet gain weight due to a slow metabolism is limited; on

average obese people have a greater energy expenditure than their thin counterparts due to the energy required to maintain an increased body mass. The incidence and prevalence of obesity continues to reach pandemic proportions and is a leading force behind the also worrisome frequency of type 2 diabetes mellitus (DM2) and cardiovascular disease (CVD), causing significant morbidity, mortality and imposing a severe economic burden to health care systems in both industrialized and non-industrialized countries [2].

According to the National Health health care systems in 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 [3].

There are important sex-related differences in the prevalence of obesity, DM2, hypertension and CVD, with premenopausal women at less risk relative to age-matched men [4], [5].

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 [6]. 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 [7].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.

Women with body weight measuring up to 30 percent more than that of the ideal are known as Obese. The body mass index (BMI), is the most popular and effective way of calculating female Obesity.

It is believed that Female Obesity has increased by 15 percent in the past decade and as a result, women have become prone to diseases like diabetes, high BP, etc. Women who have a hereditary problem of obesity need to be very careful about their food and exercise quotient. Often women find it difficult to shed post pregnancy weight which in turn leads to lifelong obesity for them. The occurrence of CVD can be reduced by decreasing the formation of atherosclerosis by lowering cholesterol levels in the blood and increase the concentration of high density lipoprotein [8].

Evidences from lipid lowering trials have clearly established that Nonalcoholic fatty liver disease (NAFLD) refers to a wide spectrum of liver diseases ranging from the most common, fatty liver (accumulation of fat in the liver, also known as steatosis), to Nonalcoholic steatohepatitis (NASH, fat in the liver causing liver inflammation), to cirrhosis (irreversible, advanced scarring of the liver as a result of chronic inflammation of the liver). All of the stages of Nonalcoholic fatty liver disease now believed to be due to insulin resistance, a condition closely associated with obesity. In fact, the BMI correlates with the degree of liver damage, that is, the greater the BMI, the greater the liver damage.

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 livers in a number of different strains and species of rodents [9]. 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, hypertrigyceridemia hyperinsulinemia, and insulin resistance [10], [11]. In addition, these diets generate pathologies independent of atherosclerosis, such as changes in fur and skin integrity, changes in plasma lipids, and hepatic steatosis [12], [13]. 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 . Dietary cholesterol has been shown to reduce fatty acid oxidation, which in turn increases the levels of hepatic and plasma triacylglycerols (TG) [14].

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 [15], [16]. The ingestion of polyunsaturated fatty acids present in vegetable oils is inversely related to the incidence of heart diseases by decreasing the cholesterol and triacyglycerol plasmatic levels. Increased adiposity and insulin resistance contribute to the progression from NASH to fibrosis through the development of a profibrotic mileau in the liver, including increased hepatocellular death [17].

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. [18]. 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 [19]. Changes in the diet in terms of fatty acid content

can have a marked effect on lipid profile of blood. The information regarding the effects of butter and estrogen with high fat diet on cardiovascular and hepatic disease is limited.

Therefore, the investigation was made by the application of high fat with estrogen to learn about any alteration in biochemical profile of blood and also in histo-texture liver and skin in mice. The whole work has been designed with the following objectives:

i.   To investigate the effects of butter and estrogen on lipid profile of blood.

ii.   To study any alteration in hepatic and cutaneous histo-texture following supplementation of butter and estrogen.

2   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 to 25 December 2013. The details of the experimental approach and methodology are presented in this chapter.

2.1   EXPERIMENTAL DESIGN

One month old 60 (30 male and 30 female) Swiss Albino mice *(Mus musculus)* with an average body weight of 15-18g were used. The mice were randomly divided into 6 equal groups of which 3 groups (M, M_1, M_2) were of male and the remaining 3 groups (F, F_1, F_2) were into the female. Each group consisted of 10 mice (n=10). All groups were supplied with standard broiler pellet (5gm/mice/day) and fresh drinking water was given ad libitum throughout the experimental period of 90 days. Group M from male and group F from female were kept as control and animals were fed with normal broiler pellet. Mice of group M_1 and F_1 were fed with 20% butter with feed. Group M_2 and F_2 were fed with conjugated estrogen at a dose rate of 10 µg/mice/day with water. Body weight, biochemical profile, was measured and histopathological observation was made in the course of study (Figure 2.1).
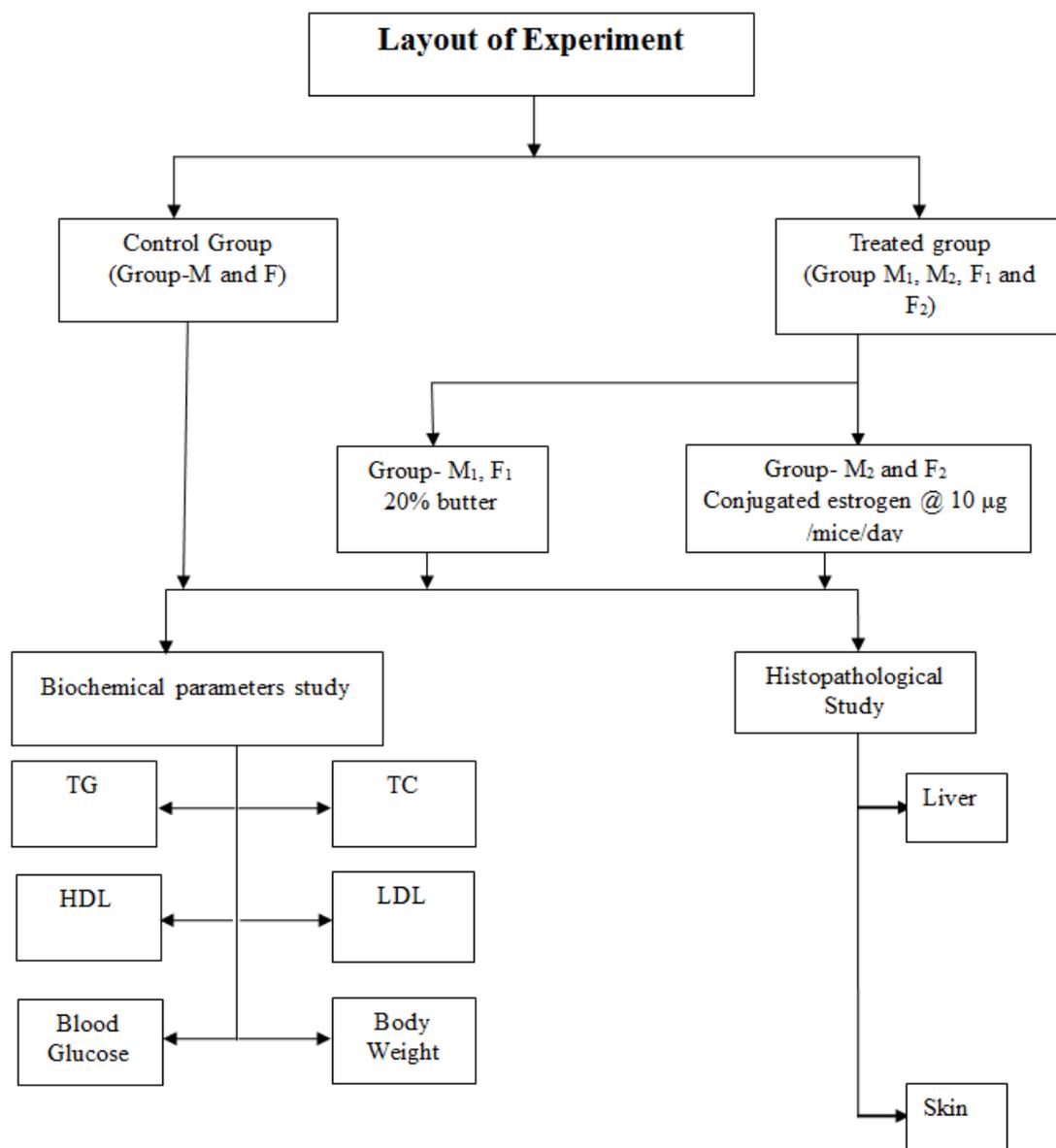
Figure 2.1: Layout of the experimental design

2.2   EXPERIMENTAL ANIMALS

The mice were purchased from ICDDR, B, 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× 11 ×7 cubic inches) wrapped with wire mesh. The cages were kept in well ventilated room at 28 ±2^{0}C and a relative humidity of 70-80% with natural day and light. The experimental laboratory was cleaned and washed at a regular interval.

2.3   EXPERIMENTAL DIET

Commercial Broiler pellet (HI-PRO-VITE feed) was collected from local feed market in Mymensingh.

Table 2.1: Composition of the HI-PRO-VITE feed (kg/100kg)

Ingredients	Amount (%)
Maize	56.70
Rice polish	16.92
Soybean meal	4.78
Fish meal	7.14
Limestone	0.50
Common salt	0.25
DCP	0.25
DL-methionine	0.06
Broiler premix	0.05
Crude protein (%)	18.40
Calcium (%)	0.09
Total phosphorus (%)	0.76
Lysine (%)	0.85
Methionine	0.38
Tryptophan (%)	0.27

Butter was collected from local market, Bangladesh Agricultural University (BAU), Mymensingh.

2.4 EXPERIMENTAL DRUGS

Tablet Estracon® (Conjugated Estrogen @0.625mg/tablet) from Renata were used to evaluate the role of high fat diet and Estrogen supplementation.

2.5 MANAGEMENT PRACTICES

The cages were kept in a well-ventilated room. In order to prevent spoilage feeds were kept in poly packed sac. The feed and fresh drinking water were supplied daily to the mice. Mice cages were cleaned regularly. Proper hygienic and sanitary measures were also taken during the experimental period. Feces were removed regularly.

2.6 COLLECTION OF BLOOD

On the 1st day (after acclimation) and at the end of the experiment, blood sample was collected by sacrificing the mice. After giving anaesthesia the mice were placed inside the air tight container one by one containing cotton soaked with chloroform. Then they were taken out of the container for blood collection. The abdominal cavity and thoracic cavity were opened surgically and blood was collected directly from the heart with the sterile syringe and needle. About 1ml of blood from the syringe was taken in the test tube containing anticoagulant (3.8% Na citrate solution) for hematological studies. The remaining amount of blood of syringe was used for collection of serum.

2.7 PREPARATION OF SERUM

2 ml of blood was collected in the sterile glass test tube. The blood containing tubes were placed in slanting position at room temperature for 1 hour. Then the clot was detached from the wall of the syringe carefully and allowed it to settle down and afterward serum was collected. Collected serum was centrifuged at 1500 rpm for 15 minutes to obtain clear serum and then stored at -20°C temperature until being used.

2.8 BIOCHEMICAL STUDIES

The biochemical parameters of serum Total Cholesterol, Triglyceride, HDL, LDL, Blood glucose.

2.8.1 DETERMINATION OF TOTAL SERUM CHOLESTEROL

The cholesterol was determined using the procedure described by [20]; 10 µl ready serum sample was taken in each cuvette (1 cm light path) with the help of micropipette. Then 1000 µl reagent was taken to each cuvette and mixed thoroughly by shaking. The cuvettes were incubated at 37^0C for 5 minutes. After incubation, each mixture was placed in the Biochemistry Humalyzer-3000 (Human type, Germany) against the blank reagent at 500 nm, 546 nm wave length. Then result was recorded from display. The result was expressed in mg/dl.

2.8.2 DETERMINATION OF TRIGLYCERIDES

The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quiononeimine formed from hydrogen peroxide, 4- aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The triglyceride of blood serum is determined by Biochemistry Humalyzer-3000 (Human type, Germany) according to the technique described by [20]. This procedure is similar to total serum cholesterol. The result was expressed in mg/dl.

2.8.3 DETERMINATION OF HDL CHOLESTEROL

Two hundred µl serum samples was mixed with 500 µl diluted precipitant in the test tube. Then the mixture was allowed to sit for 10 minutes at room temperature and then centrifuged for 10 minutes at 4000 rpm. 100 µl clear supernatant was separated within two hours in such way 20 supernatant was from 20 serum sample. 100µl supernatant was taken in the cuvette (1 cm light path) by micropipette. Then 1000 µl reagent (Cholesterol) was mixed with supernatant by shaking. After mixing the mixture was incubated in Reflectron ® Humalyzer 3000 (Human type, Germany) for 5 minutes at 37^0C. Then the mixture was placed in the Reflection ® against the blank reagent at a wave length of 500 nm. Then result was recorded in mg/dl which displayed in the Biochemistry Humalyzer-3000 (Human type, Germany). The result was expressed in mg/dl.

2.8.4 DETERMINATION OF LDL CHOLESTEROL

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.

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

2.8.5 DETERMINATION OF BLOOD GLUCOSE

Procedure

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

2.9 HISTOPATHOLOGICAL PROCEDURE

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

2.9.1 COLLECTION OF SAMPLES

On the 90th day of the experiment, liver and aorta from the experimental mice were collected by sacrificing in 10% buffered formalin and used for histopathological study.

2.9.2   PREPARATION OF 10% BUFFERED FORMALIN

Table 2.2: Preparation of 10% buffered formalin

37-40% formalin	100 ml
Distilled water	900 ml
Sodium Phosphate (monobasic)	4 gm.
Sodium Phosphate (dibasic)	6.5 gm.

The above ingredients were mixed thoroughly, preserved in an air tight container split in plastic jar @ 250ml/jar

2.9.3   CHEMICALS REQUIRED

(i) Alcohol (50%, 70%, 80%, 95% & absolute)
(ii) Chloroform
(iii) Paraffin
(iv) Xylene
(v) Distilled water
(vi) Hematoxylin
(vii) Acid alcohol
(viii) Ammonium water
(ix) Eosin

2.9.4   HISTOPATHOLOGICAL EXAMINATION PROCEDURE

Fixed tissue sections were processed for paraffin-embedding, sectioning [21]staining with Hematoxylin & Eosin stain.

2.9.5   PROCESSING OF TISSUES

The formalin fixed tissues were properly trimmed. The tissues were washed overnight under running tap water to remove formalin. The tissues were dehydrated in ascending grades of alcohol.

Table 2.3: Time required for dehydrating tissues

50% alcohol	1 hour
70% alcohol	1 hour
80% alcohol	1 hour
95% alcohol	1 hour

The tissues were cleared in 10% buffered formalin for two changes in chloroform, 1.5 hour in each. The tissues were embedded with melted paraffin wax at 56^0C for two changes, 1.5 hour in each. Paraffin block containing tissue pieces were made using templates. The tissues were sectioned with a microtome at 5 µm thickness, allowed to spread on warm water bath (40^0) containing a small amount of gelatin & taken on oil and grease free glass slides. The slides were air dried and kept in cool place until staining.

2.9.6   STAINING PROCEDURE

2.9.6.1   PREPARATION OF HARRIS HEMATOXYLIN SOLUTION

Table 2.4: Preparation of Harris hematoxylin solution

Hematoxylin crystals	5 gm.
Alcohol (100%)	50 ml
Ammonium or potassium alum	100 ml
Distilled water	100 ml
Mercuric oxide (red)	2.5 gm.

The hematoxylin was dissolved in the alcohol and the alum in the water by the aid of heat. Two solutions were thoroughly mixed and boiled as rapidly as possible. After removing from heat, mercuric oxide was added slowly and reheated to simmer unit it become dark purple. The solution was then removed from heat immediately and plunged the vessel into basin of cold water until become cool. Immediately before using, 2-4 ml of glacial acetic acid was added per 100ml of solution to increase the precision of the nuclear stain. Before using prepared solution was filtered and kept in the dark [21].

2.9.6.2   PREPARATION OF EOSIN SOLUTION (1% STOCK ALCOHOLIC EOSIN)

Table 2.5: Preparation of eosin solution

Eosin Y, water soluble	1gm
Distilled water	20 ml
Dissolved and 95% alcohol	80 ml

WORKING EOSIN SOLUTION

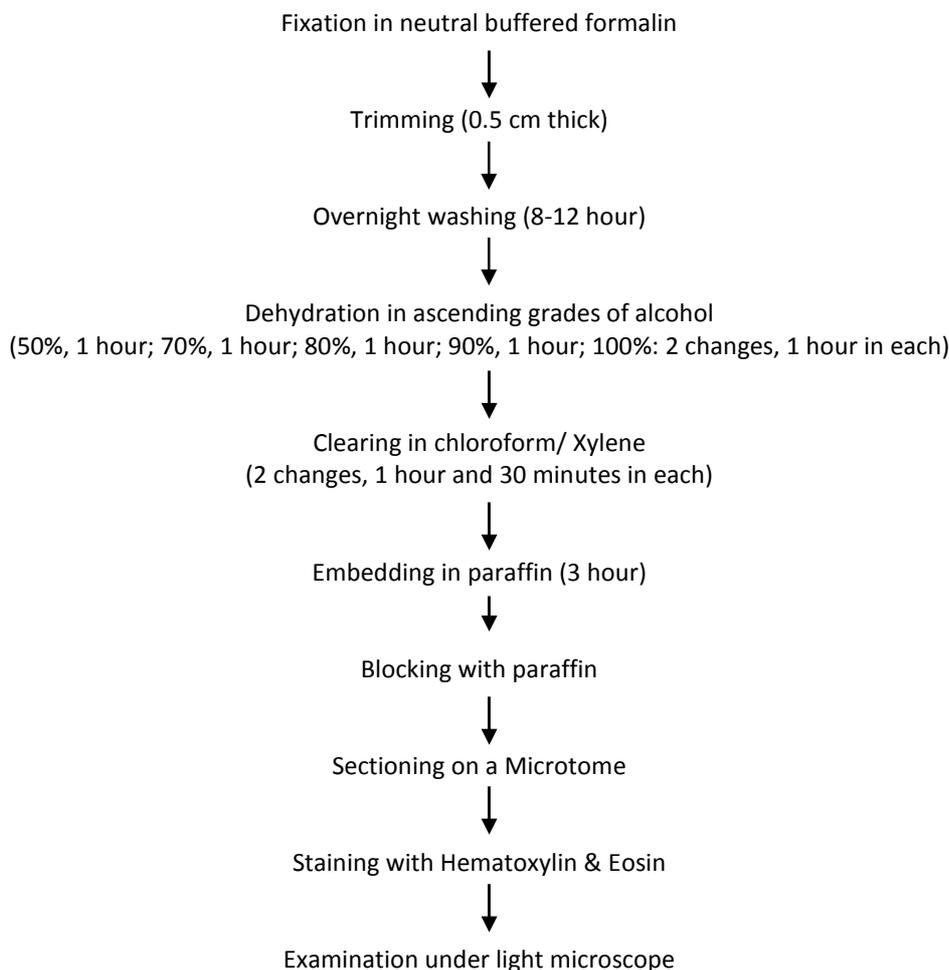| Eosin stock solution	1part
Alcohol, 80%	3 parts

Immediately before use 0.5 ml of glacial acetic acid was added to every 100 ml of stain & stirred.

2.9.6.3   ROUTINE HEMATOXYLIN & EOSIN STAINING PROCEDURE

The tissue sections were deparaffinized in 3 changes of Xyline (3 minutes in each). Rehydrations of the sectioned tissues were done through descending grades of alcohol [22].

Flow chart for Hematoxylin & Eosin staining:

95% alcohol for 2 minutes

↓

80% alcohol for 2 minutes

↓

70% alcohol for 2 minutes

↓

Distilled water for 5 minutes

↓

Staining with Harris Hematoxylin for 15 minutes

↓

Washing in running tap water for 15 minutes

↓

Differentiated in acid alcohol: 2 to 4 dip (1 part HCl in 99 parts 70% alcohol)

↓

The tissue sections were then washed in tap water for 5 minutes.

↓

Blue in ammonia water (2-3 quick dips)

↓

The sections were then stained with eosin for 1 minute.

↓

Differentiation & dehydration in 95% alcohol:3 changes (1 dips in each).

↓

The stained sections were then cleaned by 3 changes in Xyline, 5 minutes in each. Finally the sections were mounted with coverslip using DPX.

Flow chart for histopathology:

Fixation in neutral buffered formalin

↓

Trimming (0.5 cm thick)

↓

Overnight washing (8-12 hour)

↓

Dehydration in ascending grades of alcohol
(50%, 1 hour; 70%, 1 hour; 80%, 1 hour; 90%, 1 hour; 100%: 2 changes, 1 hour in each)

↓

Clearing in chloroform/ Xylene
(2 changes, 1 hour and 30 minutes in each)

↓

Embedding in paraffin (3 hour)

↓

Blocking with paraffin

↓

Sectioning on a Microtome

↓

Staining with Hematoxylin & Eosin

↓

Examination under light microscope

2.9.7   HISTOPATHOLOGICAL STUDIES AND PHOTOMICROGRAPH

The tissues were examined and photomicrographs were taken in the Anatomy and histology laboratory, BAU, Mymensingh

2.10   STATISTICAL ANALYSIS

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.

3   RESULTS

The research work was conducted in 6 groups (each group consisting 10 mice) of mice following dietary supplementation of butter and estrogen. The results of the experiment are presented under the following Tables:

Table 3.1 Effect of butter and estrogen on lipid profile in mice

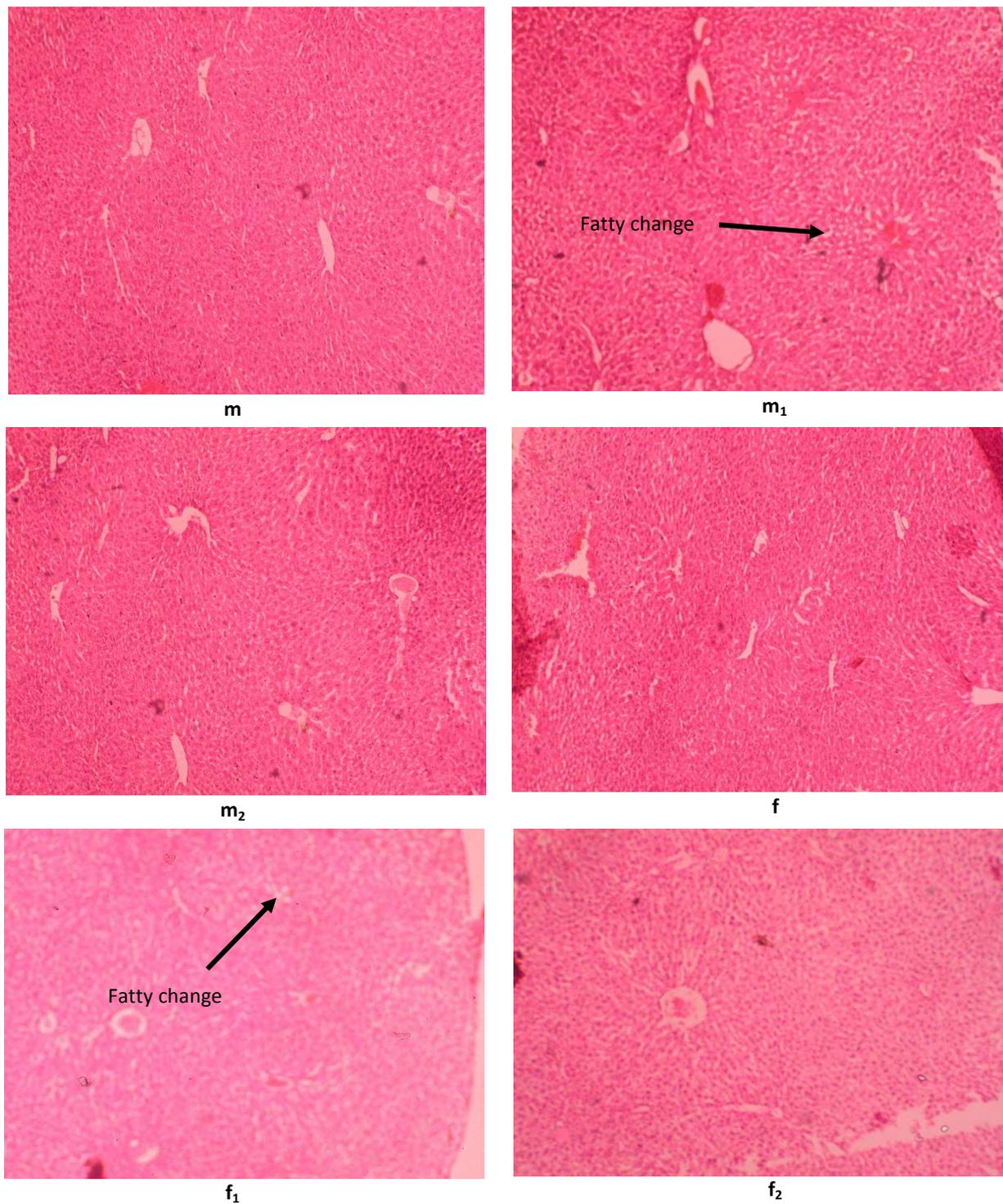| Sex	Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Blood sugar(mg/dl)	Body Weight (g)
		values	values	values	values	values	values
Male	M (Control)	48.74±1.20[c]	53.60±0.75[c]	20.05±1.04[b]	29.37±1.74[c]	48.26±1.05[b]	29.87 ±0.35[b]
	M$_1$ (Butter)	84.52±1.38[a]	103.70±0.65[a]	19.10±1.14[b]	95.71±1.78[a]	95.13±1.64[a]	37.17 ±0.57[a]
	M$_2$ (Estrogen)	55.47±1.92[b]	72.24±0.81[b]	48.81±1.15[a]	35.69±2.62[b]	47.84±1.22[b]	33.93 ±1.20[b]
Female	F (Control)	49.27±1.01[c]	54.11±0.36[c]	19.16±1.07[b]	30.89±1.86[c]	49.35±1.58[b]	30.37 ±0.65[b]
	F$_1$ (Butter)	132.38±1.89[a]	139.04±1.56[a]	19.00±1.23[c]	97.42±3.43[a]	102.92±1.14[a]	38.63±0.45[a]
	F$_2$ (Estrogen)	54.68±1.81[b]	75.63±0.98	54.14±1.15[a]	36.07±1.00[b]	48.84±1.0[b]	34.63 ±0.57[b]

3.1   HISTOPATHOLOGY

Figure. 3.1 Histopathological section of liver of mice (H & E x 400) (m) Control Male; (m$_1$) Butter treated male; (m$_2$) Estrogen treated male; (f) Control female; (f$_1$) Butter treated female; (f$_2$) Estogen treated female.
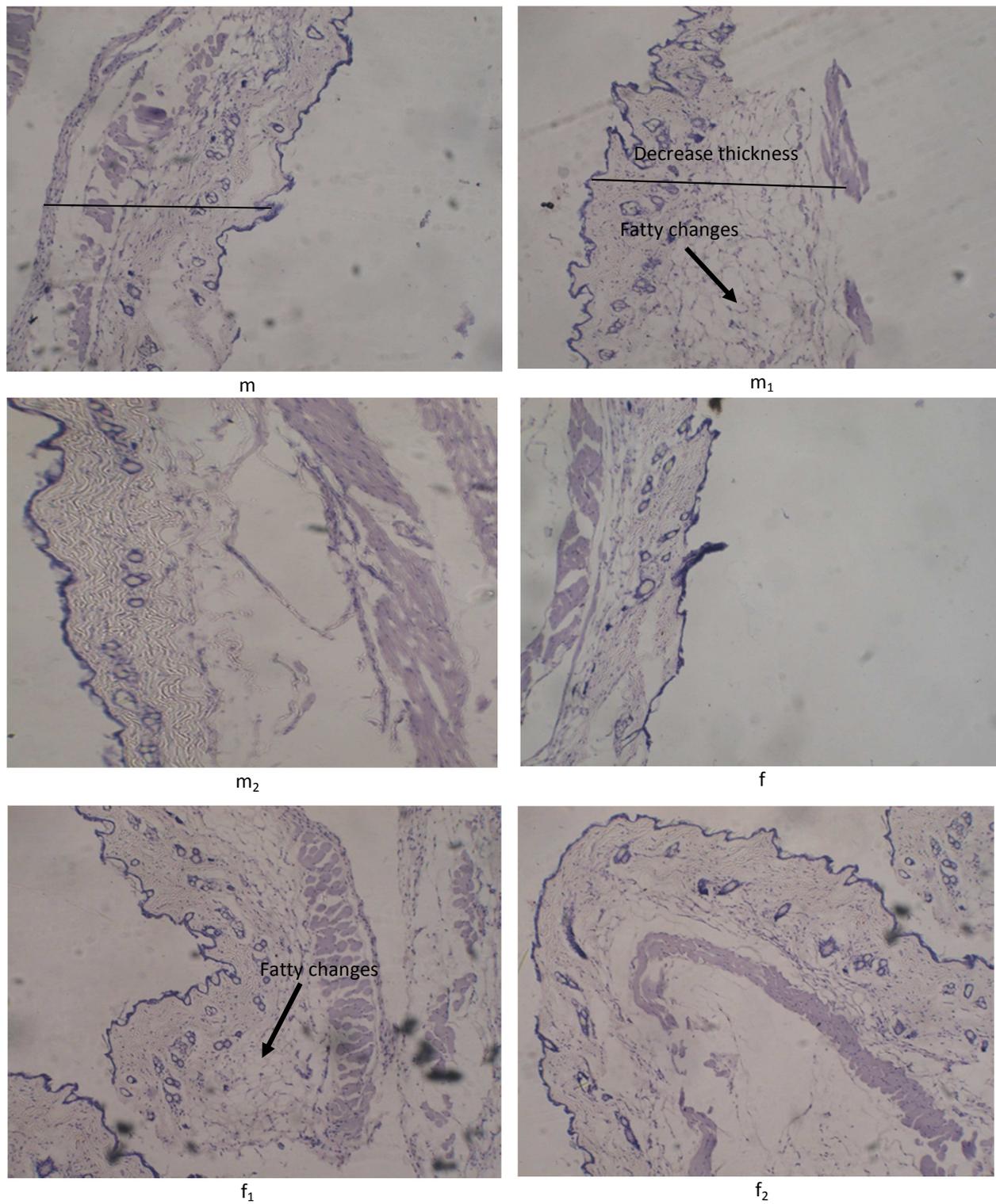
Fig. 3.2 Histopathological section of skin of mice (H & E x 400) (m) Control Male; (m$_1$) Butter treated male; (m$_2$) Estrogen treated male; (f) Control female (f$_1$) Butter treated female; (f$_2$) Estogen treated female.

4   SUMMARY AND CONCLUSION

The experiment was undertaken in order to investigate the effects of butter and estrogen supplemented diet on lipid profile, blood sugar and histopathology of liver and skin in relation to obesity of mice with a study period of 90 days. One month old 60 (30 male and 30 female) Swiss Albino mice *(Mus musculus)* with an average body weight of 15-18 g were used. The mice were randomly divided into 6 equal groups of which 3 groups (M, M_1, M_2) were of male and the remaining 3 groups (F, F_1, F_2) were into the female. Each group consisted of 10 mice (n=10). Group M and F were considered as control; Group M_1 and F_1 butter treated; Group M_2 and F_2 estrogen treated. Blood samples were collected at the beginning of the study that is on day 1 of the experiment (1 month old mice) and on day 90 i.e. at the end of the study (4 months old) by sacrificing the animals to analyze haematological parameters and blood biochemical profiles. At the end of experiment, histopathological examinations of two selected organs (liver and skin) were performed. The effects of butter and estrogen on blood sugar and lipid profiles of mice varied significantly in the present study.

Among all the treated groups, the mice of groups M_1 and F_1 (butter treated male and female respectively) exhibited significant ($P<0.01$) increase in body weight compared to the other groups. In lipid profile study, there was significant ($P<0.05$) changes in butter treated mice (M_1 and F_1). In butter treated mice, blood glucose level was increased significantly in group M_1 and F_1 compared to the other group ($P<0.01$). In histopathological examinations, significant changes were recorded in the liver and skin of butter treated groups in comparison with the mice of control group. There were fatty changes in skin but there were no tumours as found in high fat diet mice. After successfully completing the trials, among the treated groups; group F_1 and M_1 (butter treated group) showed the maximum body weight and obesity.

The present research findings help in drawing a conclusion that 20% butter supplementation would be able to cause changes in lipid profile, increased body weight and blood sugar level and would also produce degenerative changes in liver and skin.

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, amount of estrogen used, 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 in order to investigate the combined and counter effect of butter and estrogen in mice 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.

REFERENCES

[1]   C. K. Glass and J. L. Witztum, "Atherosclerosis, the road ahead", Cell 104: 503-16, 2001.
[2]   Flegal KM, Carroll MD, Ogden CL, Curtin LR, "Prevalence and trends in obesity among US adults", 1999-2008. Journal of American Medical Association. 303:235–241; 2010. [PubMed]
[3]   Flegal KM, Carroll MD, Kit BK, Ogden CL, "Prevalence of obesity and trends in the distribution of body mass index among US adults", 1999-2010. Journal of American Medical Association. 307:491–497, 2012. [PubMed]
[4]   Barrett-Connor E, Giardina EGV. Gitt AK. Gudat U. Steinberg HO. Tschoepe D, "Women and heart disease: the role of diabetes and hyperglycemia", Arch Intern Med. 164:934–942, 2004. [PubMed]
[5]   Shi H and Clegg DJ, "Sex differences in the regulation of body weight", Physiol Behav. 97:199–204; 2009. [PubMed]
[6]   Okrainec K, Banerjee DK, Eisenberg MJ, "Coronary artery disease in the developing world", American Journal of Heart 148 7-15, 2004
[7]   Kalim H, Karo-Karo S, Soerianata S, "Guidelines for treatment of dyslipidemia in coronary heart disease prevention", Persatuan Dokter Spesialis Kardiovaskuler Indonesia. Jakarta. [Indonesia], 1996.
[8]   Nogrady T. 1992. Medicinal chemistry: a biochemical approach. Penerbit ITB. Bandung. [Indonesia], 1992.
[9]   Koteish A and Diehl AM, "Animal models of steatosis", Semin Liver Disease 21 89–104, 2001.
[10]   Hartvigsen K, Binder CJ, Hansen LF, "A diet-induced hypercholesterolemic murine model to study atherogenesis without obesity and metabolic syndrome", Arteriosclerosis, Thrombosis and Vascular Biology 27 878–85, 2007.
[11]   Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass CK, "Peroxisome proliferator-activate inhibit development of atherosclerosis in LDL receptor-deficient mice" *Journal of Clinical Investigation* **106** 523–31,2000.

[12] Lichtman AH, Clinton SK, Iiyama K, Connelly PW, Libby P, Cybulsky MI , "Hyperlipidemia and atherosclerotic lesion development in LDL receptor deficient mice fed defined semipurified diets with and without cholate" Arteriosclerosis, Thrombosis and Vascular Biology 19 1938–44, 1999.

[13] Gajda A, Pellizzon M, Ricci M, Ullman E , "Diet-induced metabolic syndrome in rodent models", Animal Laboratory News 365 1415–28, 2007.

[14] Fungwe, T. V., L. M. Cagen, H. G. Wilcox, and M. Heim- berg, "Regulation of hepatic secretion of very low den-sity lipoprotein by dietary cholesterol", J. Lipid Res. 33: 179-191, 1992.

[15] Brown AS, Bakker ARG, Yellen L, "Treating patients with documented atherosclerosis to National Cholesterol Education Program - recommended low density lipoprotein cholesterol goals with atorvastatin, fluvastatin, lovastatin and simvastatin", Journal of Molecular Cell and Cardiology 32 665-92, 1998.

[16] Grundy SM, Cleeman JI, Daniels SR, "Diagnosis and management of the metabolic syndrome – An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement", Circulation 112 2735–52, 2005.

[17] Chiang DJ, Pritchard MT, Nagy LE, "Obesity, diabetes mellitus, and liver fibrosis", Gastrointestinal and Liver Physiology, 300 697-702, 2011.

[18] Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, Fraser GE, Goldbourt U, Hallmans G, "Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies". The American Journal of Clinical Nutrition, 89 1425–32, 2009.

[19] Yuan YV, Kitts DD, Godin DV, "Interactive effects of increased intake of saturated fat and cholesterol on atherosclerosis in the Japanese quail (Coturnix japonica)", British Journal of Nutrition, 80 89–100, 1998.

[20] Trinder P, "Cholesterol determination test", Clinical Biochemistry 6 24-25, 1969.

[21] Luna LG: Manual of Histologic Staining Methods of the Armed Forces   Institute of Pathology. 3rd ed. McGraw-Hill, New York, 1968.

[22] Davenpot HA: Histology and Histochemical techniques. W.B. Saunders Company, Philadelphia, 1960