RESEARCH ARTICLE OPEN ACCESS

Antihyperlipidemic Effect of Soybeans Milk (*Glycine Max)* in High-Fat Diet-Induced Hyperlipidemic Albino Rats

*Umar, A. I., Salihu, S., Muhammad, N.B. and Shafiu, A. A.

Department of Biochemistry, Sokoto State University, Sokoto, Nigeria
*Corresponding author: E - mail mamunetdaji@gmail.com Phone: +2348065310438

ABSTRACT

Hyperlipidemia is characterized by increased level of *cholesterol* or *triglyceride*-carrying *lipoproteins* in plasma, exceeding an arbitrary normal limit. This study evaluates the antihyperlipidemic effect of soybeans milk in rats. Hyperlipidemia was induced through high animal fat diet feeding for a period of four weeks. Four groups of five (5) animals each were used for the study. Group A (Control); Group B (HFD); Group C (HFD + soybeans milk [5ml/kg]) and Group D (HFD + soybeans Milk [10ml/kg]). Body weight was measured using weighing balance. Serum lipid profiles were evaluated using standard methods. Values obtained indicated decreased serum TC, TAG and LDL, with the exception of HDL in Group C and D when compared to Group B (positive control). Therefore, hyperlipidemia induced by high fat diet feeding was restored to near normal levels by soybeans milk supplementation. In light of this, soybeans milk may serve as a useful dietary intervention in the management of hyperlipidemia caused by high fat diet feeding.

Keywords: Hyperlipidemia, soybeans milk, high fat diet (HFD), TC, TG and LDL.

INTRODUCTION

Hyperlipidemia refers to high amount of lipoproteins or lipids, such as low-density lipoprotein (LDL), free fatty acids and triglyceride (TAG) in the blood (Klop *et al.,* 2013). It is a widely accepted risk factor for cardiovascular disease (Guendouzi *et al.,* 1999), atherosclerosis, ischemic cerebrovascular disease, coronary heart disease and peripheral vascular diseases (Umadevi and Anoosha, 2018). It is an abnormality characterized by elevated serum total cholesterol, serum triglyceride, low density lipoprotein-cholesterol levels and reduced high density lipoprotein-cholesterol levels (Klop *et al.,* 2013). It pose a major risk for serious diet related chronic diseases, including type 2 diabetes, cardiovascular diseases, hypertension, and stroke, as well as certain form of cancer (Hotamisligil, 2006; Xu *et al.,* 2003). The health consequences range from increase risk of premature death, to serious chronic conditions that reduce the overall quality of life Various factors such as lipid abnormalities (Castelli *et al*., 1986), oxidative stress (Yokoyama and Kawashima, 2004) and

inflammation (Hansson, 2005) have been associated with the development of atherosclerosis and cardiovascular disease. There have been reports that hyperlipidemia in humans (Beynen and Katan, 1985) as well as animals (Dietschy, 1997) is produced by the influence of dietary cholesterol. Diet is known to play a pivotal role in maintenance of ideal body weight, body fat and normal levels of blood lipids. Research reports have been demonstrated in understanding the pathophysiology of hyperlipidemia. According to LaRosa *et al*. (1990), prevention or treatment of atherosclerosis and cardiovascular diseases is significantly possible through the use of diets and drugs in targeting hyperlipidemia. On this note, this study intends to find a nutritive therapy for the management of hyperlipidemia using cheap and readily available food (soybeans) consumed in modarawwa community, Sokoto State, Nigeria.

Materials and Methods

Preparation of Soybeans milk:

Soybeans was purchased from Abu kure market Sokoto , transported to botany unit department of Biological sciences, Usmanu Danfodiyo University sokoto for identification. The butcher number (UDUTH/NHS/004) was obtained. It was finally transported to general laboratory of Biochemistry Department of Sokoto State University for the analysis. 2,500g of soybeans was sorted, washed and soaked in 4500ml of distil water for 8 hours. It was grinded and sieved to separate the liquid mixture (soybeans milk) from the debris. The soybeans milk was then incubated at 48°C.

Preparation of High Fat Diet (HFD):

High fat diet containing 46% fat was formulated using the following formula as reported by Validicantos *et al.* (2009): 46% animal fat, 24% carbohydrate, 20.3% protein, 5% fibre, 3.7% salt and 1% vitamin mixture.

Induction of Hyperlipidemia:

Hyperlipidemia was induced according to method adapted by Saad *et al*. (2011). Experimental rats received high animal fat diet (containing 46% fat) for four weeks (4weeks).

Experimental Animals:

Twenty (20) Wister Albino rats of both sexes weighing between 150-200g were used for this study. The animals were grouped into four (4) groups of five (5) rats each. They were housed in wired cages and allowed to acclimatize for seven days before the commencement of the experiment. They were fed with pelletized growers feed and allowed free access to water *ad libitum* before and during the experimental period.

Grouping of Animals:

Group A: Normal Control: They are normal rats that received normal feed

Group B: High Fat diet Untreated: They received high animal fat diet (containing 46% fat) for four weeks (4weeks) and served as hyperlipidemic untreated group

Group C: High fat diet; Treated with soybeans milk: They were administered with high animal fat diet (containing 46% fat) for four weeks (4weeks) and treated with 5ml/kg of soybeans milk

Group D: High fat diet; Treated with soybeans milk: They were administered with high animal fat diet (containing 46% fat) for four weeks (4weeks) and treated with 10ml/kg of soybeans milk

Sample Collection:

At the end of the experiment, the rats were subjected to 10 hours fast and anesthetized with chloroform vapour. 5ml blood samples were collected by cardiac puncture into labeled centrifuge tubes allowed to cloth and centrifuged at 4000 rpm for 10 minutes. 5ml of serum was pipette into labeled test tubes and stored at 4^0C for biochemical analysis.

Biochemical analysis:

The sera obtained were used for measuring serum lipid profile. Serum total cholesterol (TC) was measured by enzymatic method using Randox Kit (Allain *et al*.,1974). Serum HDL was measured by enzymatic method using Randox kit (Burstein *et al*.,1970). Serum triacylglycerol (TAG) was measured by enzymatic method using Randox kit (Tietz, 1990). LDL was calculated using Friedewald Formula (Friedewald *et al*., 1972).

Result and Discussion:

Serum Lipid Profile of High Fat Diet-Induced Hyperlipidemic Rats after Ten Weeks of Treatment with Soybeans Milk

Result of lipid profile of untreated rats and rats treated with different concentrations of soybean milk is presented in table1 below. It shows a significant decrease ($p< 0.05$) in serum cholesterol (TC) in Group C and D when compared to group B. There is no significant decrease ($p> 0.05$) in serum triglyceride in Group C when compared to group B. Serum TC, triglyceride and LDL levels are lower in group D which received a high number of dose (10ml) than group C that received 5ml of soybean milk.

TABLE 1: Serum Lipid Profile of High Fat Diet-Induced Hyperlipidemic Rats after Four Weeks of Treatment with Soya beans Milk

GROUP	TC(mol/dl)	TAG(mol/dl)	HDL(mol/dl)	LDL(mol/dl)
A	183.75±1.250[a]	110.00±4.082[a]	55.5±0.0455[a]	100.00±4.082[a]
B	273.25±1.315[b]	181.25±1.250[b]	45.5±2.021[b]	171.75±1.031[b]
C	243.5±14.506[c]	171.25±0.9265[ac]	75.00±1.080[ac]	142.0±1.082[c]
D	198.75±1.258[a]	161.25±1.258[ab]	92.5±1.190[c]	138.75±0.4787[c]

Values are mean ± SEM. Values with the same super script letter are not significantly different (p> 0.05)

Body weights of high fat diet induced hyperlipidemic rats after four (4) weeks of treatment with Soybeans milk

Results of body weight changes of normal, untreated, and treated rats are presented in table 2 below. It shows increase in body weight in group B indicating that consumption of food that is very rich in fat increases body weight. Similarly it showed decrease in body weight in groups feed with HFD and treated with soybeans milk (ie group C and D).

Table 2 Body weight of high fat diet induced hyperlipidemic rats after four (4) weeks of treatment with Soybeans milk

GROUP	WEEK 1 BW(g)	WEEK 2 BW(g)	WEEK 3 BW(g)	WEEK 4 BW(g)
A	257.5 ±3.22	262.5 ±1.47	277.5±1.44	285.5±11.81
B	281.25 ±2.31	297.5 ± 1.53	300.5 ± 13.1	310.0± 4.08
C	273.5 ±2.17	292.5 ±1.63	289.5 ±7.50	280.75.±9.43
D	272.5± 1.44	286.0±2.04	283.0 ± 10.0	276.75± 11.43

Values are mean ± SEM. BW stands for body weight

DISCUSSION

Hyperlipidemia is a strong contributor to the pathogenesis of cardiovascular diseases and other health complications associated with obesity (Klop *et al.,* 2013). This study investigates the antihyperlipidemic effect of soybeans milk in high fat diet induced hyperlipidemia in rats. Many researchers were able to induce hyperlipidemia and its associated complications using different formulae of high fat diets (Kim *et al.,* 2005; Diniz *et al.,* 2005). In the present study, hyperlipidemia was induced in rats using high fat diet containing 46% fat.

An evidence of hyperlipidemia was recorded in the present study. The results of our findings revealed that HFD feeding in rats resulted in significant (p<0.05) increase in serum levels of TAG, LDL and TC, and decreased serum levels of HDL-C in HFD fed rats (Group B) when compared with the control (Group A) (Table 1). Hyperlipidemia that occured in HFD-induced rats may be due to increase triglyceride content of the liver as a result of increased influx of non-essential fatty acids (NEFAs) into the liver (Grundy, 2004) of the rats. However, our findings have revealed significant (p<0.05) decrease in serum TC, TAG and LDL, and a significant (p<0.01) increase in serum HDL of the HFD fed rats treated with soybeans milk (Group C and D) when compared with the HFD fed untreated rats (Group B) (Table 2). The decrease in total cholesterol (TC), triglyceride (TAG), low density lipoprotein cholesterol (LDL) and increase in high density lipoprotein (HDL) in group C and D observed in this study is in accordance with findings of Ramchandran (2013), in his study on effect of soybeans milk on hyperlipideamic rat and it phamacordynamic potential. Soybeans contain many component or ingredient that have biochemical benefit which include soy protein, soy saponin, and isoflavone. Soybeans milk has the ability of lowering the level of cholesterol, triglyceride, low density lipoprotein, and increase the level of HDL. The possible machanisms of the antihyperlipidemic effect of soy protein intake may include the improvement of insulin and glucagon ratio, which is involved in lowering fatty acid biosynthesis in the liver through reducing the gene expression of sterol regulatory element binding protein (SREBP)-1.

The results of the present study revealed significant (p<0.05) rise in body weight of high fat diet (HFD) fed untreated rats (Group B) in comparism with the control (Group A) (Table 2). This finding is in agreement with that of Laila *et al.,* (2012), suggesting that high fat diet feeding causes significant increase in body weight in rats. This may be attributable to excessive accumulation of fat in body tissues, or increase in tissue mass as a result of fat accumulation. However, our findings also revealed that camel milk supplementation reversed this change in body weight (Table 2) in group C and D respectively due to the intake of soybeans milk.

CONCLUSION

The abnormalities (hyperlipidemia and high body weight) induced by high fat diet feeding were reversed by soybeans milk supplementation in albino rats. Therefore, soybeans milk may serve as a useful dietary intervention in the management of hyperlipidemia caused by high fat diet feeding.

REFRENCES

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. and Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, 20; 470.

Beynen, A. C., and Katan, M. B. (1985). Reproducibility of the variations between humans in the response of serum cholesterol to cessation of egg consumption. *Atherosclerosis*, 57(1);19-31.

Burstein, M., Scholnick, H.R. and Morfin, R. (1970).Rapid method for the isolation of lipoproteins from human serum byprecipitation with polyanions. *Journal of Lipid Reearch*, 11: 583-595.

Castelli, W. P., Garrison, R. J., Wilson, P. W., Abbott, R.D., Kalousdian, S. and Kannel, W. B. (1986) Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *Journal of American Medical Association,* 256 (20); 2835-8.

Dietschy, J. M. (1997). Theoretical considerations of what regulates low-density-lipoprotein and high-density-lipoprotein cholesterol. *American Journal of Clinical Nutrition*, 65; 1581S–9S.

Diniz, Y.S., Faine, L.A., Galhardi, C.M., Rodrigues, H.G., Ebaid, G.X., Burneiko, R.C., Cicogna, A.C., and Novelli, E.L. (2005). Monosodium Glutamate in Standard and High-Fiber Diets. Metabolic Syndrome and Oxidative Stress in Rats. *Journal of Nutrition*, 21;749.

Friedewald, W.T., Levy, R.I., Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry,* 18; 499–502.

Grundy, S. M. (2004). Metabolic complications of obesity. Obesity, Metabolic Syndrome, and Cardiovascular Disease. *Journal of Clinical Endocrinology & Metabolism*, 89(6);2595– 2600.

Guendouzi, K., Jaspard, B., Barbaras, R., Motta, C., Vieu, C., Marcel, Y., Chap, H., Perret, B., Collet, X. (1999). Biochemical and physical properties of remnant-HDL2 and of pre beta 1-HDL produced by hepatic lipase. *Biochemistry*. 38**,** 2762–2768.

Hansson, G. K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *New England Journal of Medicine*, 353(4);429-30

Hotamisligil, G.S. (2006). Inflammation and metabolic disorders. *Nature*, 444; 860–867

Kim, J.H., Hahm, D.H., Yang, D.C., Kim, J.H., Lee, H.J., and Shim, I. (2005). Effect of Crude Saponin of Korean Red Ginseng on High-Fat Diet-Induced Obesity in Rat. *Journal of Pharmacological Science*, 97;124.

Klop, B., Elte, J.W. and Cabezas, M.C. (2013). Dyslipidemia in obesity: Mechanisms and potential targets. *Nutrients,* 5; 1218–1240

Laila, A.E., Samah, E. and Nashwa, E. (2012). Nerve conduction velocity of static nerve in high fat diet induced obesity in rats: Effects of corn oil and omega 3 fatty acids supplement. *Life Science Journal,* 9(3); 2301-2312.

LaRosa, J.C., Hunninghake, D., Bush, D., Criqui, M.H., Getz, G.S., Gotto, A.M. Jr, Grundy, S.M Rakita, L., Robertson, R.M. and Weisfeldt, M.L. (1990). The cholesterol facts. A summary of the evidence relating dietary fats, serum cholesterol, and coronary heart disease. A joint statement by the American Heart Association and the National Heart, Lung, and Blood Institute. The Task Force on Cholesterol Issues, American Heart

Association. *US National Library of Medicine National Institutes of Health*, 81(5);1721-33.

Ramachandran, S., Rajani, Sanjay, A. S., and Dhanaraju, M. D. (2013) Effect of Soya milk on Dyslipidaemic rats and its Pharmacodynamic Potential with Statins. *Journal of Advanced Pharmacy Education & Research*, 3(3);289-293

Saad, A. N., Hala, E. H., and Amal, A. B. (2011). Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. *Diabetology and Metabolic Syndrome,* 3;17

Tietz, N. W. (1990). Serum triglyceride determination. In: Clinical guide to laboratory tests, second edition, W. B. Suaunders co, Philadelphia, USA, Pp 554-556

Umadevi, R. and Anoosha, T. (2018). Evaluation of Oryza sativum for Anti hyperlipidemic Activity in rats. *International journal of engineering science and computing*, 8 (1); 15922-15926.

Valdicantos, P., Matute, P., and Martinez, A. (2009). Obesity and oxidative role of antioxidant supplementation. *Journal of Review of Clinical Investigation*, 61; 127-127.

Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., Xu, A., Wang, Y., Keshaw, H., Xu, L.Y., Lam, K.S.,Cooper, G.J. (2003). The fat-derived hormone adiponectin alleviates alcoholic and non-alcoholic fatty liver diseases in mice. *Journal of Clinical Investigation*, 112; 91–100.

Yokoyama, M. and Kawashima, S. (2004). Dysfunction of endothelial nitric oxide synthase and atherosclerosis. Arterioscler Thromb Vasc Biol., 24 (6): 998-1005.

