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Effect of Mulberry (*Morus alba* L.) Leaves Extract on the Secretion and Content of Triglyceride in the Chicken Hepatocytes Primary Culture

¹Abdonnaser Mohebibi, ¹Zohre Khaki, ²Farzad Asadi, ²Malihe Pourkabir and ³Mehrdad Modirsanei
¹Department of Clinical Sciences, ²Department of Biochemistry,
³Department of Animal Nutrition and Breeding, School of Veterinary Medicine,
University of Tehran, Tehran, Iran

Abstract: In present study, twelve 6 week age chickens were decapitated; liver extracted, sliced and cultured as primary culture. Effects of hydro extract of mulberry leaf on TG secretion and hepatic TG were determined. Data indicated that hydro extract of mulberry leaf extract decreased triglyceride secretion in a dose dependent manner (as much as 82, 76 and 67% in response to 0.075, 0.05 and 0.015% of hydro extract after 48 h incubation, respectively). Moreover, at 0.075 concentration it decreased TG content as much as 43% after 12 h incubation. Mulberry leaves contain some inhibitory components for accumulation and secretion of TG in chicken hepatocytes.

Key words: Triglyceride, chicken hepatocyte, mulberry leaf extract

INTRODUCTION

Excessive accumulation of lipids in the adipose tissue of modern lines of broilers is a major concern for producers, because most fat depots are lost during evisceration of the carcass or processing of the meat. TG storage in avian adipose tissue depends on the availability of plasma lipid substrate, mainly very low density lipoprotein (VLDL), originating from either the diet or lipogenesis in the liver (Hermier, 1997). In young broiler chickens approaching market weight, about 80-85% of the fatty acids that accumulate in the adipose tissue are derived from plasma lipids (Griffin, 1992). Because commercial avian breeds are usually fed lipid-poor diets the liver plays a key role in providing lipids destined to be used by all tissues. Therefore any attempt to modify these metabolic processes must take into consideration the specific features of lipid metabolism in birds (Hermier, 1997).

In the recent years, several plant flavonoids were identified as hypolipidemic agents in tissue culture, animals and humans (Jahromi *et al.*, 1993; Arai *et al.*, 2000; Wilcox *et al.*, 2001; Cassachi *et al.*, 2004; Li *et al.*, 2006). They inhibit the activity of a number of lipogenic enzymes, including hydroxymethylglutaryl (HMG) CoA reductase, acyl CoA:cholesterol acyltransferase (ACAT), microsomal triglyceride transfer protein (MTP) and CoA:diacylglycerol acyltransferase (DGAT) (Wilcox *et al.*, 2001; Theriault *et al.*, 2000; Casaschi *et al.*, 2004).

Mulberry (*M. alba* L.) leaves containing many nutritional components are the best feed for silkworms and they have been used in traditional Chinese medicine as an antihyperglycemic. Major flavonoids have been extracted from the mulberry leaves are quercetin and its glycosylated forms specially rutin (Jia *et al.*, 2001; Katsube *et al.*, 2006). Andallu (2001) have showed oral administration of mulberry leaves powder cause decrease in blood and urine glucose, TG, LDL-cholesterol and VLDL-cholesterol and Fatty acids in type-2 diabetic patients.

The aim of the present study was to examine the effects of mulberry leaves extract on TG secretion and content in chicken hepatocytes primary culture.

MATERIALS AND METHODS

Mulberry leaves extract preparation: Mulberry leaves were collected from campus of school of Veterinary Medicine, University of Tehran on 12 June, 2004. The leaves were washed three times with tap water. Shade dried leaves were ground to powder. Powder (1 g) was extracted in a Soxhlet extractor with 100 mL distilled water 1 h and filtered. The extract was evaporated to dryness. After weighing, the dry extract was dissolved in distilled water to make 40% (w/w) stock solution.

Cell culture: Isolation and Culture of chicken hepatocytes were performed as previously described by

Tarlow and Watkins (1977) by some modification. Briefly, the liver of twelve 6 week-old roosters was extracted after 12 h fast (water *ad libitum*). The livers were minced finely. Thirty milliliter of 1.5 IU mL⁻¹ collagenase (type IV) solution and 2.5 mL of 400 mM glucose were added to the minced tissue. The tissue suspension was incubated for 20 min at 37°C in a gyratory shaker set at 130 oscillations min⁻¹. The obtained suspension containing dissociated cells was filtered through 50 and 100-mesh nylon screen. The cell pellet was subjected to a procedure for lysing erythrocytes by washing with 20 mL of RBC lysing buffer (8.26 g of NH₄Cl, 1.0 g of KHCO₃, 0.037 g of Na₂-EDTA in 1 L of distilled water). The cell pellet was washed with serum free DMEM medium to remove the RBC derbies. Hepatocytes resuspended in DMEM medium supplemented with FBS (5%), penicillin (100 IU mL⁻¹), streptomycin (100 µg mL⁻¹), insulin (10 µg mL⁻¹). Cell viability, estimated by the trypan blue exclusion test or the well-preserved refringent shape, was always greater than 80%. For experiments, cells were plated out at a density of 1.5×10⁶ cells cm⁻² (500 µL well⁻¹) in 17 mm dishes (24-well plates) and preincubated for 3 h at 37°C in 5% CO₂. Cells were then changed to 1% BSA in serum-free medium with the indicated treatments and incubated for the different times at 37°C.

Lipid extraction and TG quantification: At the end of the incubation period, the cells were placed on ice and the medium was collected. The cells were washed three times with 1 mL ice-cold PBS and the cell lipids were extracted with 2 mL heptane/isopropanol (3:2, v/v). After 4 h, the extracts were removed and the cells were washed once with an additional 1 mL heptane/isopropanol (3:2, v/v) and added to the previous extract (Cianflone *et al.*, 1992). The soluble cell protein was dissolved in 1 mL 0.1 N NaOH and total cell protein was measured by Bradford method, using

BSA as a standard (Bradford, 1976). Media lipids were extracted by adding 1 mL of chloroform/methanol (80/20, v/v) methanol (80/20, v/v), decanting for 10 min and the organic phase was separated for TG assay. TG was measured by the method of Neri and Frings (Neri and Frings, 1973), both in cell extract and media.

Statistical analysis: All values are presented as mean±SD. Statistical significance was tested by one-way ANOVA, SigmaStat 2.03.

RESULTS

Cell viability was determined using the trypan blue exclusion test and cell protein measurement. There was no difference in cell viability between control cells and cells treated with different doses of mulberry leaves extract in different incubation times (data not shown).

Effect of mulberry extract on TG secretion: Different doses of mulberry leaves extract, 0.015, 0.05 and 0.075% decreased TG secretion from chicken hepatocytes compare to control group after 12, 24 and 48 h incubation in a dose dependent manner (p<0.001). 10⁻⁹ M glucagon also decreased TG secretion in different incubation times. However 0.075% MLE caused more decreasing in TG secretion compare to 10⁻⁹ M glucagon (p<0.005) (Table 1).

Effect of mulberry leaves extract on cell TG content: 0.075% MLE and 10⁻⁹ M glucagon decreased chicken hepatocytes TG content after 12, 24 and 48 h incubation times (p<0.001). No significant differences observed between effect of 0.075% MLE and 10⁻⁹ M glucagon cell TG content (p>0.05). However, 0.015 and 0.05% extracts showed any effect on cells TG content (p>0.05) (Table 2).

Table 1: Effect of MLE and 10⁻⁹ M glucagon on TG secretion in chicken hepatocytes primary culture. Values were expressed as µmol L⁻¹

Time (h)	Control	MLE			Glucagon 10 ⁻⁹ M
		0.015%	0.05%	0.075%	
12	607.38±112.88	272.01±16.73*	185.97±18.53*	125.07±24.85*	210.49±2.77*
24	913.02±172.18	303.60±52.76*	217.56±28.13*	161.18±19.29*	251.39±46.29*
48	951.92±184.32	428.06±77.28*	332.17±60.75*	214.25±38.20*	285.99±22.84*

Values were presented as mean±SD. *The mean value differs significantly from the control in one way-ANOVA (p<0.001, n = 12)

Table 2: Effect of MLE and 10⁻⁹ M glucagon on TG content in chicken hepatocytes primary culture. Values were expressed as µmol mg⁻¹ cell protein

Time (h)	Control	MLE			Glucagon 10 ⁻⁹ M
		0.015%	0.05%	0.075%	
12	123.44±20.24	130.7±17.94	125.16±18.38	83.93±12.15*	81.46±2.9*
24	139.99±23.02	126.6±29.4	132.2±18.85	90.84±12.99*	71.14±6.7*
48	142.43±17.85	140.1±15.82	143.55±21.26	98.67±13.63*	96.17±8.97*

Values were presented as mean±SD. *The mean value differs significantly from the control in one way-ANOVA test (p<0.001, n = 12)

DISCUSSION

Several flavonoids dramatically reduce apoB secretion in cell culture. A number of lipogenic enzymes are involved in the mechanism of action, including HMG CoA reductase, ACAT, MTP and DGAT (Casaschi *et al.*, 2002; Theriault *et al.*, 2000; Wilcox *et al.*, 2001). However, the enzymes predominantly responsible for the assembly of apoB-Lp in the presence of flavonoids have not been thoroughly investigated. Borradaile *et al.* (2002) made progress in this area by ruling out ACAT activity and cholesterol ester (CE) availability in the regulation of apoB-Lp by naringenin, a citrus flavonoid, in epG2 cells. Casaschi *et al.* (2004) showed that Chalcone Xanthohumol a plant polyphenol, decrease apoB secretion in Hep G2 cells via the decreasing of microsomal triglyceride transfer protein (MTP) and CoA:diacylglycerol acyltransferase (DGAT) activity.

The present study provides evidence that mulberry leaves extract have ability for influencing TG metabolism in the chicken hepatocytes. We have shown a dose dependent inhibitory effect for MLE on TG secretion and cell content ($p < 0.001$). Mulberry leaves contain five flavonol glycosides: rutin, isoquercitrin, quercetin 3-(6-acetylglucoside), astragalol and kaempferol 3-(6-acetylglucoside). From these compounds rutin and Quercetin 3-(6-malonylglucoside) are the most ones in the mulberry leaves (Katsube *et al.*, 2006). In literature review, we found any *in vitro* study on the effects of whole mulberry leaf extract on the synthesis and secretion of TG in hepatocytes. However Andallu *et al.* (2001) showed oral administration of mulberry leaves powder has a dual effect (decreasing of TG synthesis and increasing of VLDL removing) on serum TG levels in diabetics. In this regard, Casaschi *et al.* (2002) showed that quercetin inhibited TG synthesis in intestinal cell-line, CaCO₂, in part, via DGAT activity. In this respect, Theriault *et al.* (2000) showed that taxifolin, a plant flavonoid, decreased TG synthesis and secretion in HepG2 cells through inhibition of DGAT and MTP activity.

In present study, although all doses of MLE decreased TG secretion from chicken hepatocytes, TG content only was affected by 0.075% of MLE. Nakamura *et al.* (2000) reported decrease in serum TG followed by oral administration of rutin in rats without alteration in hepatic TG content. Moreover, we have shown that 0.075% of MLE's effect on the TG secretion and content was greater than 10^{-9} glucagon ($p < 0.001$). Tarlow and Watkins (1977) showed glucagon decrease TG secretion and synthesis via suppression of *de novo* lipogenesis in chicken hepatocytes.

Future studies will need to examine the mechanism of mulberry leaves extract induced changes in TG secretion and content of chicken hepatocytes deduce if the effect is due to decreased *de novo* fatty acid synthesis or increased β -oxidation of fatty acids before administration to rooster *in vivo*. However these data suggest that mulberry leaves extract administration may be an alternative method to reduce fat mass and providing increased meat yield.

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REFERENCES

- Andallu, B., A.V. Suryakantham, B.L. Srikanthi and G.K. Reddy, 2001. Effect of mulberry (*Morus indica* L.) therapy on plasma and erythrocyte membrane lipids in patients with type 2 diabetes. Clin. Chim. Acta, 314: 47-53.
- Arai, Y., S. Watanabe, M. Kimira, K. Shimoi, R. Mochizuki and N. Kinai, 2000. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J. Nutr., 130: 2243-2250.
- Borradaile, N.M., L.E. de Dreu, P.H. Barrett and M.W. Huff, 2002. Inhibition of hepatocyte apoB secretion by naringenin: Enhanced rapid intracellular degradation independent of reduced microsomal cholesteryl esters. J. Lipid Res., 43: 1544-1554.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye-binding. Anal. Biochem., 72: 248-257.
- Casaschi, A., Q. Wang, K. Dang, A. Richards and A. Theriault, 2002. Intestinal apolipoprotein B secretion is inhibited by the flavonoid quercetin: potential role of microsomal triglyceride transfer protein and diacylglycerol acyltransferase. Lipids, 37: 647-652.
- Casaschi, A., G. Maiyoh, B. Rubio, R. Li, K. Adeli and A. Theriault, 2004. The chalcone xanthohumol inhibits triglyceride and apolipoprotein B secretion in HepG2 Cells. J. Nutr., 134: 1340-1346.
- Cianflone, K., S. Dahan, J.C. Monge and A.D. Sniderman, 1992. Pathogenesis of carbohydrate-induced hypertriglyceridemia using HepG2 cells as a model system. Arthro and Throm, 12: 271-277.

- Griffin, H.D., K. Guo, D. Windsor and S.C. Butter, 1992. Adipose tissue lipogenesis and fat deposition in linear broiler chicken. *J. Nutr.*, 122: 363-368.
- Hermier, D., 1997. Lipoprotein Metabolism and Fattening in Poultry. *J. Nutr.*, 127: 805-808.
- Jahromi, M.A. and A.B. Ray, 1993. Antihyperlipidemic effect of flavonoids from *Pterocarpus marsupium*. *J. Nat. Prod.*, 56: 989-994.
- Jia, Z., M. Tang and J. Wu, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.*, 64: 555-559.
- Katsube, T., N. Imawaka, Y. Kawano, Y. Yamazaki, K. Shiwaku and Y. Yamane, 2006. Antioxidant flavonol glycosides in mulberry (*Morus alba* L.) leaves isolated based on LDL antioxidant activity. *Food Chem.*, 97: 25-31.
- Li, L., P. Stillemark-Billton, C. Beck, P. Bostrom, L. Andersson, M. Rutberg, J. Ericsson, B. Magnusson, D. Marchesan, A. Ljungberg, J. Boren and S.O. Olofsson, 2006. Antioxidant effect of saponin: Potential action of a soybean flavonoid on glucose tolerance and risk factors for atherosclerosis. *Int. J. Food Sci. Nutr.*, 56: 79-85.
- Nakamura, Y., S. Ishimitsu and Y. Tonogai, 2000. Effects of quercetin and rutin on serum and hepatic lipid concentrations, fecal steroid excretion and serum antioxidant properties. *J. Health Sci.*, 46: 229-240.
- Neri, P. and C.S. Frings, 1973. Improved method for determination of triglyceride in serum. *Clin. Chem.*, 19: 1201-1203.
- Tarlow, D.M., P.A. Watkins, R.E. Reed, R.Z. Miller, E.E. Zwergle and M.D. Lane, 1977. Lipogenesis and the synthesis and secretion of very low density lipoprotein by avian liver cells in non proliferating monolayer cell culture. Hormonal effects. *J. Cell. Biol.*, 73: 322-353.
- Therriault, A., Q. Wang, S.C. Van Iderstine, B. Chen, A.A. Franke and K. Adeli, 2000. Modulation of hepatic lipoprotein synthesis and secretion by taxifolin. A plant flavonoid. *J. Lipid Res.*, 41: 1969-1979.
- Wilcox, L.J., N.M. Borradaile, L.E. de Dreu and M.W. Huff, 2001. Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. *J. Lipid Res.*, 42: 725-734.