Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 11, 2014

Original Article

GAMMA-ORYZANOL HAS AN EQUIVALENT EFFICACY AS A LIPID-LOWERING AGENT COMPARED TO FIBRATE AND STATIN IN TWO DYSLIPIDEMIA MICE MODELS

ANTONIO C. V. A. FILHO[1], MARIA I. F. GUEDES[1*], LUIS S. F. DUARTE[1], ABELARDO B. M. LIMA-NETO[1], LUIZ-CLAUDIO CAMERON[2], ADRIANA BASSINI[2], ICARO G. P. VIEIRA[3], TIAGO S. MELO[1], LIA MALMEIDA[1], MARIA G. R. QUEIROZ[4]

[1]Human Biochemistry Laboratory, State University of Ceará, Av. Paranjana, nº 1700, Campus do Itaperi, Fortaleza, CE, Brazil - CEP: 60740-903, [2]Laboratory of Protein Biochemistry, Federal University of State of Rio de Janeiro, Av. Pasteur nº296, Urca, Rio de janeiro, Brazil - CEP: 22290-260, [3]Technological Development Park, Av. Humberto Monte, 2977 - Bl. 310, Campus do Pici/UFC, Fortaleza, CE, Brazil – CEP: 60450-000, [4]Department of Clinical and Toxicological Analysis, Federal University of Ceará, RuaCapitão Francisco Pedro nº 1210 Fortaleza, CE, Brazil - CEP: 60430-370.
Email: izabel.guedes@uece.br.

Received: 28 Aug 2014 Revised and Accepted: 29 Sep 2014

ABSTRACT

Objective: A substantial fraction of the population is intolerant or does not respond well to the recommended treatments for dyslipidemia. The purpose of this study was to evaluate the efficacy of gamma-oryzanol (γ-ORZ) treatment in acute and long-term mouse experimental models of dyslipidemia in comparison to Gemfibrozil and Simvastatin.

Methods: For the acute dyslipidemia-induced model, dyslipidemia was induced in 40 mice using a single intra-peritoneal administration of Triton WR-1339. For the long-termmodel, dyslipidemia was induced in 24 mice using a hypercholesterolemic diet over 14 days. Thereafter, animals were divided into different groups of treatment, and orally received treatments with gamma-oryzanol (5, 25, 50mg. kg^{-1}), gemfibrozil or simvastatin. For biochemical analysis, glucose, total cholesterol and triacylglycerols were measured. Body weight and net food intake was registered weekly, and urea, creatinine, AST and ALT levels were evaluated. The data were analyzed by analysis of variance (ANOVA), followed by the Student-Newman-Keuls method,and p value of less than 0.05 was considered significant.

Results: Only the highest dose of γ-ORZ exhibited significant protective effects. Gamma-oryzanol and Gemfibrozil treatments reduced total cholesterol and triacylglycerols levels in a similar manner in the acute model. In the second model, γ-ORZ and simvastatin treatments reduced glucose and total cholesterol levels in the same way. In addition, the administration ofγ-ORZ did not cause any adverse events, or significantly altered hepatic enzymes levels, plasmatic urea or creatinine concentrations.

Conclusion: The results of this study suggest that gamma-oryzanol acts as a potential lipid-lowering agent, reducing triglycerides and total cholesterol in dyslipidemia-induced models.

Keywords: Dyslipidemia, Gamma-oryzanol, Statin, Fibrate, Cholesterol, Triglycerides, Glucose, Lipid-lowering.

INTRODUCTION

Dyslipidemia is considered a major risk factor for cardiovascular disease (CVD)[1, 2], which is the leading cause of years of life lost (YLL)worldwide[3]. Statins and fibrates are the first-line drugs for treatment of dyslipidemia [4, 5],and have been used for decades as lipid-lowering agents to improve several lipid and lipoprotein parameters[6, 7]. However, a substantial fraction of the population is intolerant or do not respond appropriately to these drugs [8, 9]. Myopathy, hypersensitivity skin reactions, headaches, pancreatitis, hepatitis, sexual dysfunction, and gastrointestinal and sleep disturbances are common adverse effects related with the use of these drugs [10]. Thus, the development of new therapeutic agents with equivalent effects remains important. Natural compounds, such as phytosterol and oryzanol, have been shown to exhibit potent cholesterol-lowering effects in previously reported animal and human studies [11-16]. Rice bran oil (RBO) is not commonly used oil worldwide, but is in steady demand as a so-called "healthy oil" in Asian countries, particularly India. Gamma-oryzanol (γ-ORZ)is a major component of RBO, and it is a mixture of ferulic acid esters of triterpene alcohols, such as cycloartenol (106 mg%) and 24-methylene cycloartanyl (494 mg%)[17, 18].

It has been reported to produce both hypocholesterolemic and hypolipoproteinemic activities, to reduce the risk of CVD and to possess anti-oxidative properties [19], but no studies to date have compared its lipid-lowering effect with currently used drugs. The purpose of this study was to evaluate the efficacy of γ-ORZ treatment in acute and long-termmouse experimental models of dyslipidemia in comparison to two commonly used drugs (Gemfibrozil and Simvastatin) and with a view to developing an alternative treatment.

MATERIALS AND METHODS

Experimental animals

Male Swiss mice weighing 25-30 g were obtained from the Central Animal House of The Federal University of Ceará and the Department of Physiology and Pharmacology. Animals were kept in propylene cages at a room temperature of 24 ± 2ºC and on a 12 h light/dark cycle with food and water provided *ad libitum* unless otherwise noted. The mice were fed with a standard laboratory chow(Nuvilab® CR1, Brasil) for two weeks for environmental adaptation (control diet, Table 1).

All experimental protocols were approved by the Federal University of Ceará Institutional Committee on Care and Use of Animals for experimentation, (protocol no. 90/10) and in accordance with the guidelines of the National Institutes of Health, Bethesda, MD.

Chemicals

Tween 80, Triton WR-1339 and γ-oryzanol were purchased from Sigma-Aldrich Co. (St. Louis, MO - USA). Simvastatin and gemfibrozilwere purchased from Merck & Co., Inc. (Whitehouse Station, NJ - USA).

Study design

For the acute dyslipidemia-induced model, dyslipidemia was induced using a single intra-peritoneal administration of Triton WR-

1339 (400 mg. kg^{-1}), as previously reported [20, 21]. 48 animals were used. The control group (CTL) received a single intra-peritoneal administration of 0,9% NaCl solution (10 µL. g^{-1}). Thereafter, animals were divided into6 groups (n = 8 each) and orally received the following treatments: Control (vehicle); T1339 (vehicle); GEMF (gemfibrozil100 mg. kg^{-1}); 5γ-ORZ (γ-oryzanol 5 mg. kg^{-1}); 25γ-ORZ (γ-oryzanol, 25 mg. kg^{-1}); and 50γ-ORZ (γ-oryzanol 50 mg. kg^{-1}). The groups received the treatment three times: 1 hour before Triton WR-1339 administration and 22 and 46 hours after induction. Gamma-oryzanol and gemfibrozil were suspended in 3% (v/v) Tween 80 and then in water. The mice were fasted for 8 h before blood sampling. 24 h and 48 h following the Triton induction, blood samples were collected and centrifuged (15 min, 2,400 x g, RT) to obtain plasma.

For the long-term dyslipidemia-induced model, dyslipidemia was induced using a hypercholesterolemic diet over 14 days, as reported by Wilson, Nicolosi [22]. 32 animals were used. Animals were dividedinto4 groups (n = 8 each) in accordance with their total cholesterol levels and were treated for 2 months as follows: CTL (normal diet + vehicle, p. o.), HCD (hypercholesterolemic diet + vehicle, p. o.),50γ-ORZ (hypercholesterolemic diet+ γ-oryzanol 50 mg. kg^{-1}, p. o.) And SIMV (hypercholesterolemic diet+ simvastatin 20 mg. kg^{-1}, p. o.). The concentrations chosen for γ-ORZ were based on the results observed in the acute model. Gamma-oryzanol and SIMV were suspended in 3% (v/v) Tween 80 in water. HCD controls received the same vehicle. After each month of treatment, the animals were fasted for 8 h, and then blood samples were collected for biochemical analysis.

Table 1: Diet Formulations

Diet component	Control Diet		Hypercholesterolemic Diet	
	g%	Kcal%	g%	kcal%
Protein	22	25	19,5	19
Carbohydrate	57	65	51	51
Fat	4	10	13,5	30
Cholesterol	0	0	1,1	0
Vitamin, mineral, other	9,5	0	8,5	0
Humidity	7,5	0	6,4	0

For biochemical analysis, glucose, total cholesterol and triacylglycerols were measured using commercially enzymatic kitsGlicose GOD, ColesterolLiquiform and TriglicéridesLiquiform (LabtestDiagnóstica, Brazil). In addition to these,body weight, net food intake was registered weekly and urea, creatinine, AST and ALT levels were measured by commercial kitsUréia CE, CK-NAC Liquiform, AST/GOT Liquiform and ALT/GPT Liquiform (LabtestDiagnóstica, Brazil), respectively.

Statistical analysis

The results are presented as the mean ± standard error of the mean (SEM). The data were analyzed by analysis of variance (ANOVA), followed by the Student-Newman-Keuls method. P value of less than 0.05 was considered significant.

RESULTS

Intra-peritoneal injection of Triton WR-1339 clearly induced dyslipidemia, characterized by an increase in blood glucose, total cholesterol (TC) and triacylglycerols (TG).24 h after induction, there was a statistically significant increase in blood glucose (70 %), TC (310 %) and TG (4925 %) (Figure 1A) in the T1339 group compared to control.

Among doses of γ-ORZ tested (5, 25 and 50 mg. kg^{-1}), only the highest dose exhibited significant protective effects. Mice treated with 50 mg γ-ORZ showed a statistically significant (25 %) reduction in blood glucose levels when compared to the T1339 group, whereas there was no change in the GEMF group. Both 50 γ-ORZ and GEMF significantlyreducedthe TC and TGin comparison to the T1339 group (Figure 1B and 1C). Even at 48 h after induction, the T1339 group showed higher levels of GLU, TC and TG when compared to the CTL group. In addition, the 50γ-ORZ group exhibited significantly reduced levels of all the parameters, approaching levels similar to those measured in the CTL group. Fig. 1

After the first month of the hypercholesterolemic diet-induced dyslipidemia model, the HCD group showed a statistically significant increase in glucose levels (20 %) compared to animals that received a normal diet (CTL). HCD efficiently increased cholesterolemia levels in all groups (47 %) compared to the CTL group. However, somewhat unexpected was the observation that triglyceride levels were reduced in the HCD group compared to CTL.

The 50γ-ORZ-treated animals showed a statistically significant reduction of 22 % in cholesterolemia levels when compared to the HCD mice, reaching levels similar to those in the CTL mice. In addition, the hypercholesterolemic diet nearly doubled (188 %) cholesterolemia in the HCD group. SIMV effectively reduced the total cholesterol (14 %) compared to the HCD group. Mice receiving the 50γ-ORZ treatment exhibited a greater reduction in total cholesterol of around30%.

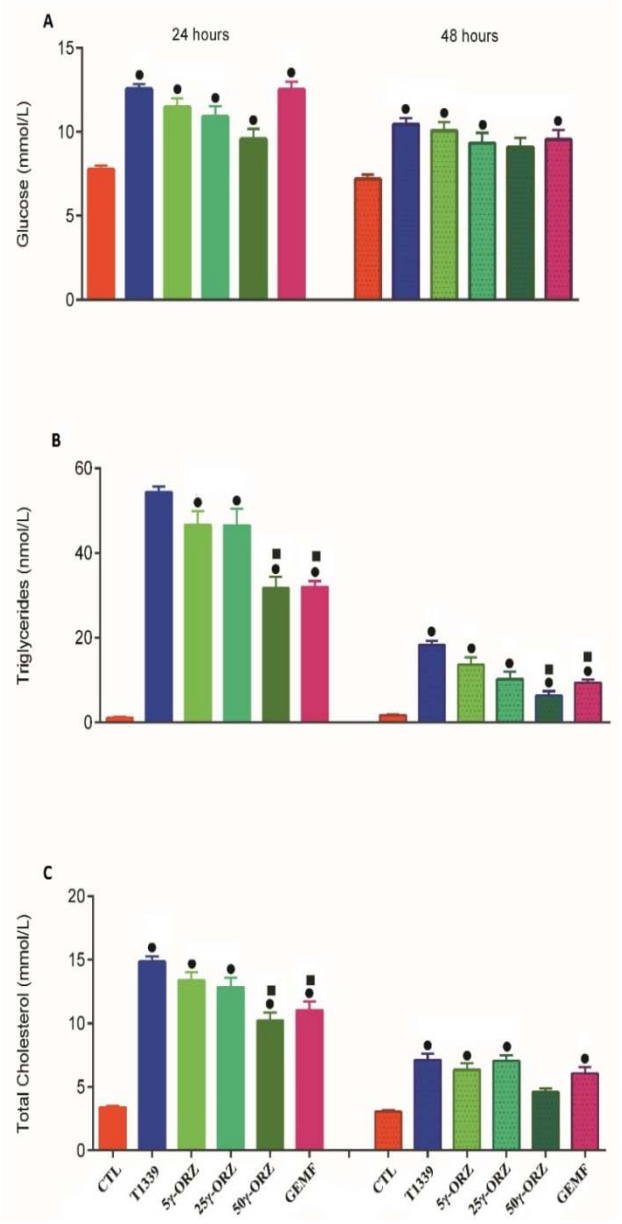

Fig. 1: γ-ORZ reduces triglycerides and total cholesterol similarly to the fibrate drug Gemfibrzil. A) Glucose, B) TG and C) TC

Values represent the mean ± SEM (n=8). γ-Oryzanol(γ-ORZ); gemfibrozil(GEMF). The groups that statistically differed from CTL and from T1339 were marked as (●)and (■) respectively.

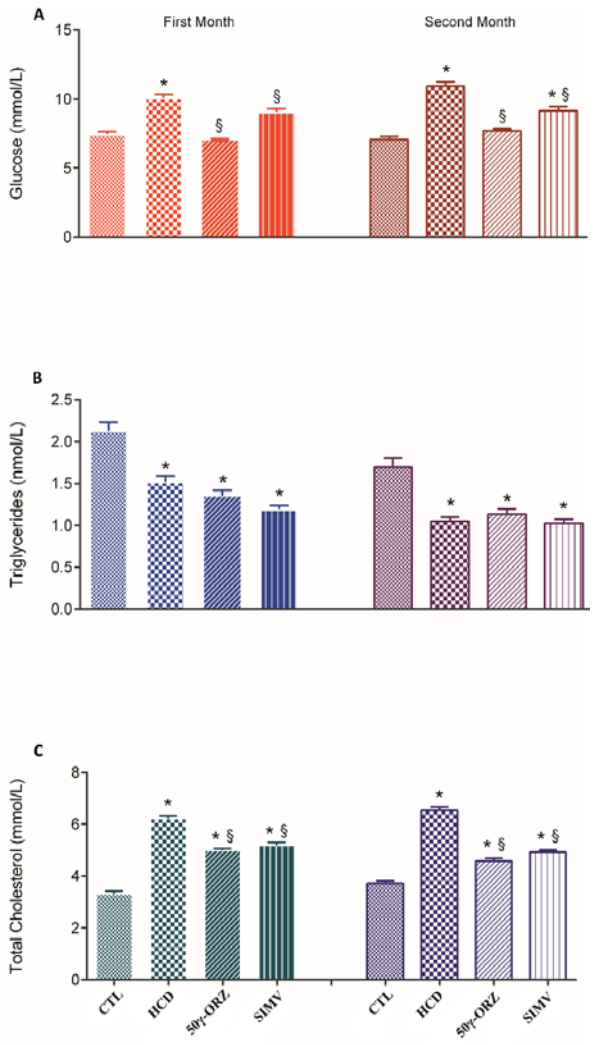

Fig. 2: γ-ORZ reduces glucose and total cholesterol similarly to the drug simvastatin. Control diet (CTL); hypercholesterolemic diet (HCD); 50 mg. kg^{-1} γ-oryzanol dose (50γ-ORZ); simvastatin (SIMV). Each value represents the mean ± SEM. The groups that statistically differed from CTL and from HCD were marked as (*) and (§), respectively.

There were no statistically significant differences in final body weights among the groups. However, an increase in the net food consumption in animals receiving the HCD was observed compared to those who received the CTL. No side effects were observed in any of the groups. The urea plasmatic levels were significantly higher in the HCD mice when compared to the CTL. Nevertheless, this increase in urea levels was reversed by the γ-ORZ and SIMV treatments. No significant changes were observed in the levels of AST, ALT or creatinine in the studied groups (data not shown).

DISCUSSION

The study presented here sought to evaluate the efficacy of γ-ORZ as a lipid-lowering drug, and compared it to existing drugs commonly used to treat dyslipidemia. Results presented here clearly show that γ-ORZ efficiently reduced blood triglycerides level and total cholesterol in dyslipidemia models.

To evaluate the acute effects of γ-ORZ on cholesterolemia and triacylglycerolemia, dyslipidemia was induced with Triton WR1339. This nonionic detergent acts to prevent the catabolism of triacylglycerol-rich lipoproteins by lipoprotein lipase (LPL)[23, 24] and is frequently used for the *in vivo* determination of triacylglycerol production, and VLDL secretion or clearance rate[20]. Using this established model[20, 23, 24], gamma-oryzanol was compared with Gemfibrozil, which is the recommended lipid-lowering agent for treatment of severe hypertriglyceridemia [25, 26]. Results showed that both treatments reduced total cholesterol and triacylglycerols levels in a similar manner.

To evaluate the efficacy of regular supplementation of γ-ORZ, dyslipidemia was also induced through a hypercholesterolemic diet and its effects were compared with Simvastatin, which is the recommended drug in the treatment of isolated hypercholesterolemia [25, 26]. Results showed that both treatments reduced glucose and total cholesterol levels in a similar manner. Interestingly, all groups that received the hypercholesterolemic diet showed a significant reduction in the triacylglycerol levels compared to the normal diet group. A similar effect has been previously observed in coconut oil fed animals, that showed a significant reduction in the serum and tissue triacylglycerol levels and a decrease in the activity of glucose-6-phosphatase dehydrogenase [27]. However, no mechanism has been suggested and more research is needed to explain this finding. Regardless of this accidental finding, results presented here clearly showed that total cholesterol was significantly reduced by both γ-ORZ and simvastatin to a similar extent.

These findings are in line with numerous previous studies reporting several physiological effects associated with γ-ORZ. These include the reduction of plasma cholesterol, the reduction of hepatic cholesterol biosynthesis, the reduction of high-fat diet induced cardiovascular disease, and the increase offecal excretion of biliary acids [28-30]. Several mechanisms have been proposed to explain these effects. For exampleMakynen, Chitchumroonchokchai [31] have suggested that the hypocholesterolemic activity of γ-ORZ is due in part to impaired apical uptake of cholesterol into enterocytes and perhaps a decrease in HMG-CoA reductase activity. Indeed, elucidating the exact mechanisms involved in the lipid-lowering activity of γ-ORZ may be valuable for designing future pharmacologically active agents to tackle dyslipidemia.

The results presented here also showed γ-ORZ to be safe. Its administration did not cause any adverse events or significantly altered hepatic enzymes levels (ALT and AST), plasmatic urea or creatinine concentrations. However, the potential side effects of gamma oryzanol are not yet known and more research is necessary to establish toxicological and side effects of this natural compound.

This study is somewhat limited by the reduced number of doses of gamma-oryzanol employed. Nevertheless, to our knowledge, this is the first attempt to compare the efficacy ofγ-ORZ with the current standard treatments for the management of dyslipidemia.

CONCLUSION

The results of this study suggest that50 mg. kg^{-1}gamma-oryzanol acts as a potential lipid-lowering agent in a manner equivalent to Simvastatin and Gemfibrozil, reducing triglycerides and total cholesterol at dyslipidemia-induced models. Whilst additional studies are needed to prove its safety, γ-oryzanol has potential tobe used as a complementary or alternative treatment for those patients who cannot achieve desired targets or who are intolerant to existing drugs.

ACKNOWLEDGMENT

The authors would like to thank OsnatHakimi for valuable comments and English language review. Andthisworkwassupportedby Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Banco do Nordeste (BNB) andCoordenação Nacional de Desenvolvimento Tecnológico e Científico (CNPq).

CONFLICT OF INTERESTS

There is no conflict of interest in this paper.

REFERENCES

1. Gotto AM Jr, Moon JE. Management of cardiovascular risk: the importance of meeting lipid targets. Am J Cardiol 2012;110(1 Suppl):3A-14A.

2. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, *et al*. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364(9438):937-52.
3. World Health Organization. World Health Statistics 2014. Geneva: WHO Press; 2014 [cited 2014];Available from: http://apps.who.int/iris/bitstream/10665/112738/1/9789240692671_eng. pdf?ua=1.
4. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, *et al*. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376(9753):1670-81.
5. Rached FH, Chapman MJ, Kontush A. An overview of the new frontiers in the treatment of atherogenic dyslipidemias. Clin Pharmacol Ther 2014;96(1):57-63.
6. Moutzouri E, Kei A, Elisaf MS, Milionis HJ. Management of dyslipidemias with fibrates, alone and in combination with statins: role of delayed-release fenofibric acid. Vasc Health Risk Manag 2010;6:525-39.
7. Katsiki N, Nikolic D, Montalto G, Banach M, Mikhailidis DP, Rizzo M. The role of fibrate treatment in dyslipidemia: an overview. Curr Pharm Des 2013;19(17):3124-31.
8. Santos RD, Waters DD, Tarasenko L, Messig M, Jukema JW, Chiang CW, *et al*. A comparison of non-HDL and LDL cholesterol goal attainment in a large, multinational patient population: the Lipid Treatment Assessment Project 2. Atheroscler 2012;224(1):150-3.
9. Arca M, Pigna G. Treating statin-intolerant patients. Diabetes Metab Syndr Obes 2011;4:155-66.
10. Ewang-Emukowhate M, Wierzbicki AS. Lipid-lowering agents. J Cardiovasc Pharmacol Ther 2013;18(5):401-11.
11. De Graaf J, De Sauvage Nolting PR, Van Dam M, Belsey EM, Kastelein JJ, Haydn Pritchard P, *et al*. Consumption of tall oil-derived phytosterols in a chocolate matrix significantly decreases plasma total and low-density lipoprotein-cholesterol levels. Br J Nutr 2002;88(5):479-88.
12. Hakala P, Lampi A-M, Ollilainen V, Werner U, Murkovic M, Wähälä K, *et al*. Steryl phenolic acid esters in cereals and their milling fractions. J Agric Food Chem 2002;50(19):5300-7.
13. Trautwein EA, Schulz C, Rieckhoff D, Kunath-Rau A, Erbersdobler HF, de Groot WA, *et al*. Effect of esterified 4-desmethylsterols and-stanols or ′4,4 -dimethylsterols on cholesterol and bile acid metabolism in hamsters. Brit J Nutr 2007;87(03):227.
14. Maud N Vissers, Peter L Zock, Gert W Meijer, Katan MB. Effect of plant sterols from rice bran oil and triterpene alcohols from sheanut oil on serum lipoprotein concentrations in humans. Am J Clin Nutr 2000;72:5.
15. Rong N, Ausman L, Nicolosi R. Rice bran oil decreases plasma LDL cholesterol by inhibiting dietary-cholesterol absorption. Faseb J 1994;8(4):A162.
16. Kahlon TS, Chow FI, Sayre RN, Betschart AA. Cholesterol-lowering in hamsters fed rice bran at various levels defatted rice bran and rice bran oil. J Nutr 1992;122:513-9.
17. Metwally AM, Habib AM, Khafagy SM. Sterols and triterpene alcohols from rice bran oil. Planta Med 1974;25(1):68-72.
18. Norton RA. Quantitation of steryl ferulate and p-coumarate esters from corn and rice. Lipids 1995;30(3):269-74.
19. Scavariello EMS, Arellano DB. Gamma-oryzanol: an important component in rice brain oil. Arch Latinoam Nutr 1998;48(1):7-12.
20. Abe C, Ikeda S, Uchida T, Yamashita K, Ichikawa T. Triton WR1339, an inhibitor of lipoprotein lipase, decreases vitamin e concentration in some tissues of rats by inhibiting its transport to liver. J Nutr 2007;137(2):345-50.
21. Sikarwar MS, Patil MB. Antihyperlipidemic activity of Salacia chinensis root extracts in triton-induced and atherogenic diet-induced hyperlipidemic rats. Indian J Pharmacol 2012;44(1):88-92.
22. Wilson TA, Nicolosi RJ, Woolfrey B, Kritchevsky D. Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters. J Nutr Biochem 2007;18(2):105-12.
23. Otway S, Robinson DS. The effect of a non-ionic detergent (Triton WR1339) on the removal of triglyceride fatty acids from the blood of the rat. J Physiol 1967;190:309-19.
24. Otway S, Robinson DS. The use of a non-ionic detergent (Triton WR1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. J Physiol 1967;190:321-32.
25. Jellinger PS, Smith DA, Mehta AE, Ganda O, Handelsman Y, Rodbard HW, *et al*. American association of clinical endocrinologists' guidelines for management of dyslipidemia and prevention of atherosclerosis. Endocr Pract 2012;18(Suppl 1):1-78.
26. Sposito AC, Caramelli B, Fonseca FA, Bertolami MC, Afiune Neto A, Souza AD, *et al*. [IV Brazilian Guideline for Dyslipidemia and Atherosclerosis prevention: Department of Atherosclerosis of Brazilian Society of Cardiology]. Arq Bras Cardiol 2007;88 Suppl 1:2-19.
27. Kim SJ, Han D, Moon KD, Rhee JS. Measurement of superoxide dismutaselike activity of natural antioxidants. Biosci Biotechnol Biochem 1995;59:822-6.
28. Seetharamaiah GS, Chandrasekhara N. Comparative hypocholesterolemic activities of oryzanol, curcumin and ferulic acid in rats. J Food Sei Technol 1993;30:249-52.
29. Nakayama S, Manabe A, Suzuki J, Sakamoto K, Inagaki T. Comparative effects of two forms of γ-Oryzanol in different sterol compositions on hyperlipidemia induced by cholesterol diet in rats. Japan J Pharmacol 1987;44:135-43.
30. Son MJ, Rico CW, Nam SH, Kang MY. Effect of oryzanol and ferulic acid on the glucose metabolism of mice fed with a high-fat diet. J Food Sci 2011;76(1):H7-H10.
31. Makynen K, Chitchumroonchokchai C, Adisakwattana S, Failla M, Ariyapitipun T. Effect of gamma-oryzanol on the bioaccessibility and synthesis of cholesterol. Eur Rev Med Pharmacol Sci 2012;16(1):49-56.