Esculetin Modulation of Lipid Profiles in White New Zealand Rabbits: A Case-Control Study

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Abstract:

Background: Dyslipidemia is a major condition that closely correlates with cardiovascular disease. Several kinds of medication, such as statins, fibrates, and so on, could control this condition. However, all of these medications have several adverse effects. Esculetin is a natural derivation produced from coumarin. It could be helpful as a new drug in the treatment of dyslipidemia as it has promising anti-inflammatory, anti-diabetic, anti-adipogenic, and antineoplastic effects.

Methods: We evaluated the impact of esculetin among 28 rabbits that got a high-fat diet. The rabbits were weighted and categorized randomly into three groups. Then lipid profile was checked and high-fat diet was started. The third group also got esculetin beside the diet. After sixty days (on day sixty-one), lipid profiles were checked from our subjects. The data were evaluated with IBM SPSS version 26.0.

Result: the second and the third group showed a significant increase in total cholesterol levels after intervention induced (P=0.001 and 0.007, respectively). However, although the second and third groups did not differ prior to the intervention, the total cholesterol level means of the third group was significantly lower than the second group after the treatment with esculetin (696 mg/dL and 290 mg/dL, respectively) (P=0.08 and 0.022, respectively).

Conclusions: Our study illustrates that the subjects taking esculetin have lower total cholesterol and triglyceride levels. Moreover, it could prevent weight gain in the rabbits received high-fat diet.

Keywords: esculetin; dyslipidemia; cardiovascular disorders; coumarin

Introduction:

Background:

Dyslipidemia is an important condition closely related to major cardiovascular events and stroke. Cardiovascular diseases account for the highest proportion of mortality and morbidity worldwide; consequently, better control of its risk factors, such as dyslipidemia, can significantly enhance global public health. During recent decades the global burden of dyslipidemia slightly increased, and disability-adjusted life years (DALYs) of high low-density lipoprotein (LDL) Cholesterol plasma levels have mounted in recent years [1]. The American Heart Association (AHA) describes dyslipidemia as an increase in fasting lipid profile, which contains a total cholesterol level equal to or above 240 mg/dl, LDL cholesterol more

than 160 mg/dl, and triglyceride (TG) above 200 mg/dl, or high-density lipoprotein (HDL) cholesterol level less than 40 mg/dl. Due to the importance of dyslipidemia in cardiac events calculating the 10-year risk for stroke and heart diseases is mandatory [2-4].

The goal of treatment in dyslipidemia is to reduce cardiovascular events and normalize LDL cholesterol levels. In addition, triglyceride and HDL cholesterol levels are other treatment priorities. Various drugs are prescribed in dyslipidemia treatment, for instance, statins, fibrate, bile acid sequestrants, ezetimibe, niacin, and omega-3. Statins, such as atorvastatin, rosuvastatin, and fluvastatin which inhibit the hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, are used solely in most cases as the initial treatment to reduce blood LDL cholesterol and total cholesterol levels while others are usually prescribed as combination therapy. Previously several complications reported in using the drugs existed for the treatment of dyslipidemia. Myositis and abnormal liver function tests are the most serious adverse effects of statins. Gemfibrozil and fenofibrate are categorized in the fibrate group, which has an extreme effect on very-low-density lipoprotein (VLDL) cholesterol levels and are widely used in patients with a high level of triglyceride. The most prevalent complication of fibrates are renal and gallbladder diseases, skin rash, and nausea. Bile acid sequestrants are almost safe drugs since they have no absorption through the gastrointestinal tract and reduce cholesterol levels by inhibiting the reabsorption of bile acids in the ileum; however, because of their unpleasant taste and gastro-intestinal side effects, most of the patients discontinue their medications. Ezetiamab and niacin are other medications that could cure dyslipidemia, although liver problems are possible to happen [3-5] (Figure. 1)
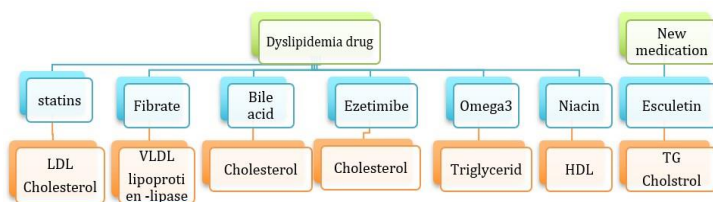

Figure 1: Effect of different drugs on lipid profile

Considering these wide ranges of adverse effects, finding a drug with higher efficacy and lower side effects, is necessary. Esculetin is a natural derivative of coumarin with a chemical formula of $C_9H_6O_4$, which can be extracted from various herbal plants such as the *Fraxinus* genus, *Citrus limon*, and *Osbeck* [6]. When the hydroxyl group was added to the 6th and 7th carbon molecule of coumarin, esculetin is made. It has effects on several cell-molecular pathways, for instance, gene-regulating processes, protein expressing, and cell signaling pathways [6-8]. Several studies have shown that esculetin has analgesic [9], anti-inflammatory [10], anti-diabetic [10], anti-adipogenic [11], antineoplastic [12], and anti-oxidant effects [6, 13-15].(Figure. 2). Also, this substance inhibits various enzymes such as Cyclooxygenase [6], lipoxygenase-5 [17,18], Catechol-O-methyltransferase [19], and NADPH oxidase [20].
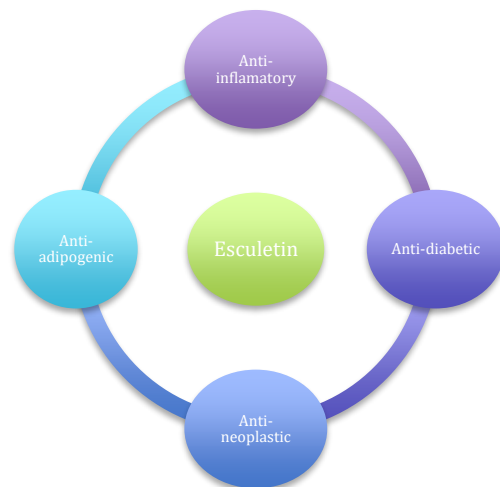

Figure 2: Different mechanisms that esculetin could induce in natural cells.

Lipoxygenase catalyzes molecular oxygen in unsaturated fatty acids in the form of 1-4-cis result in oxidation of lipoproteins which leads to atherosclerosis [21,22]. Esculetin inhibited the lipoxygenase process. Thus, it could help to prevent atherosclerosis. Zhang, L. et al. illustrated that this natural dihydroxy coumarin could reduce LDL cholesterol levels and increase HDL cholesterol thus, the rate of atherosclerosis and hyperlipidemia would diminish. Esculetin, by LDL choleterol oxidation and inhibiting matrix metalloproteinase-6 (MMP-6), has been shown promising effects in treating atherosclerosis [6]. Pullaiah, C. P. et al., in their study on mammalian subjects, demonstrated that esculetin is able to exert cardio-protective effects in myocardial infarction [15]. However, evidence regarding its prophylactic effect on cardiovascular diseases is lacking. From this issue, our study is novel. In the present study, due to lack of trials on the protective impact of esculetin, we aimed to assess the efficacy of esculetin in treatment of dyslipidemia, a major risk factor of cardiovascular events, in New Zealand rabbits so that further studies with human subjects would be conducted to better address this issue.

Material and Methods:

This interventional study followed an animal clinical trial protocol, and all the procedures were reviewed and approved by the Committee for Animal Experiments at the Jahrom University of Medical Sciences. The trial was performed on thirty male white New Zealand rabbits weighing approximately between 1.8 kilograms (kg) and 2.3 kg and was bought from the Pasteur institution in Tehran, Iran. The rabbits were housed individually under the controlled condition at a temperature of $22\pm2°$centigrade, constant humidity, and light and dark cycles of twelve hours.

Also, the rabbits had been fed a regular standard diet for sixty days. Besides, rabbits with the relatively same amount of weight are kept together. The subjects were randomly divided into three various groups; however, unfortunately, two subjects deceased before the initiation of the experiments. Thus, twenty-eight rabbits entered the study; eight rabbits were allocated to the control group (Grope 1),

ten subjects to a "high-cholesterol diet without esculetin" group (Grope 2), and the rest of them to the "high cholesterol diet plus esculetin" group (Grope 3). Also, the rabbits were weighed before and after the intervention. Previous to the intervention implementation, rabbits' ears were shaved with razor baldness and numbed with Xylocaine 10mg spray. Then blood samples were taken from rabbits' ear arteries with an insulin needle. We chose rabbits' ear vessels owing to many studies recommending that these vessels are extremely easy to access and that they could not harm rabbits [3]. After that, the fasting lipid profile was sent to the laboratory for the measurement of TG, total Cholesterol, LDL cholesterol, and HDL cholesterol.

Then, the experimental diet started for the rabbits. The initial group of eight rabbits was fed via a syringe with 3cc/day of olive oil; the second group of ten rabbits was given 3cc/day of olive oil and 0.5 g/Kg/day of cholesterol, while the third group was fed with 3cc/day of olive oil, 0.5 g/Kg/day of cholesterol, and 10 mg/Kg of our experimental drug, esculetin. Finally, on day sixty-one, the rabbits were weighed again. A blood sample was taken from the subject's other side ear and referred to the same laboratory for measurement evaluation. We should mention that 12 hours of fasting were achieved prior to the last samplings from the subjects.

A photometric method using Biotik, Iran kits had used for the measurement of TG, total Cholesterol, HDL cholesterol, and LDL cholesterol serum levels. The data was entered into IBM SPSS version 26.0. the difference between the mean weights of the groups was evaluated using a one-way Analysis of Variances (one-way ANOVA) test and Tukey post hoc test. Paired T-test was used to assess the efficacy of esculetin in the case group.

Results:

The subjects' pre-intervention weight in all three groups did not have any significant difference, which addresses that the subjects disturb in all groups equally (P=0.9). In contrast, the weight of the second group, which got high cholesterol diet prominently, increased, and it is obvious due to the diet (P= 0.02). Interestingly, the third group, which dieted with our experimental drug esculetin did not show a significant increase in weight which means that esculetin did not lead to weight gain and also it might have a preventable effect (P=0.71). Furthermore, the comparison between groups 2 and 3 showed that our intervention has a meaningful effect on preventing weight gain in the group which takes esculetin (p=0.01).

Additionally, the second and the third group showed a significant increase in total cholesterol levels after intervention induced (P=0.001 and 0.007, respectively) due to their high-cholesterol diet. However, although the second and third groups did not differ prior to the intervention, the total cholesterol level means of the third group was significantly lower than the second group after the treatment with esculetin (696 mg/dL and 290 mg/dL, respectively) (P=0.08 and 0.022, respectively).

The post-intervention triglyceride level of the second group depicts significant increases with a mean of 110.6 mg/dL (P=0.032), while the third group showed a non-significant decrease in their

triglyceride level with a mean of 53.1 mg/dL (P=0.236). In other words, in a group, which got esculetin, the difference between pre and post-intervention means and variances has a prominent deviation (P=0.05).

In addition, although in both intervention groups, the HDL cholesterol levels mounted (P=0.003 and 0.008, respectively), the increases in HDL cholesterol levels were more prominent in the second group. It might relate to the significant differences regarding the higher HDL cholesterol levels of the second group prior to the intervention(P=0.012). The HDL level means of the second group before intervention was 17.1 mg/dL; however, for the third group was 12.4 mg/dL.

The outcomes of esculetin therapy on LDL cholesterol levels were more favorable since both intervention groups illustrated a prominent surge in their LDL cholesterol levels (P=0.002 and 0.002, respectively). However, after the intervention, the third group had lower LDL choleterool levels, but the difference was not statistically significant (P=0.132).

It should mention that the pre-interventional measurement of weight, total cholesterol, HDL cholesterol, and LDL cholesterol levels among all three groups have no significant differences (P= 0.90, 0.08, 0.07, and 0.93, respectively). It depicted that the disturbance of the rabbits is the same in all three groups, and our post-intervention data are not related to chance. Table 1 summarizes the results of pre-intervention and post-intervention measurements of the weight and lipid profile of the subjects.

Variable		Group 1	Group 2	Group 3	p-value (groups 2 and 3)
Weight	Before	2034±163	2115±194	2105±172	0.904
	After	2333±166	2419±287	2136±187	0.018
P-value (paired T test)		0.001	0.024	0.719	
Total Cholesterol	Before	32.38±9.5	35.4±12.2	31.4±8.55	0.08
	After	25.37±7.85	696±457.74	290.1±230±43	0.022
P-value (paired T test)		0.159	0.001	0.007	
triglyceride	Before	54.38±14.74	45.2±11	67.8±11.22	<0.001
	After	37±5.53	110.6±79.76	53.1±33.37	0.05
P-value (paired T test)		0.009	0.032	0.263	
HDL	Before	88.31±14.3	17.1±7.47	12.4±1.01	0.079
	After	82.6±13.38	55.4±30.5	26±13.2	0.012
P-value (paired T test)		0.595	0.003	0.008	
LDL	Before	14.75±4.98	12.5±2.32	12.6±2.79	0.939
	After	8.38±3.38	227.159±2.76	136.87±3.93	0.132
P-value (paired T test)		0.036	0.002	0.002	

Table 1: The weight and lipid profile of the subjects in the study

Discussion:

In the present article, we evaluated the effect of esculetin in the enhancement of lipid profile in the murine specimens. The samples were allocated to three groups fed with high-lipid diets, with one of them consuming esculetin as well. We found that esculetin was significantly effective in the reduction of weight, triglyceride, and

total cholesterol. It could also decrease the level of LDL cholesterol and HDL cholesterol, but the clinical outcome was not statistically significant.

These days, owing to a sedentary lifestyle, high-fat diet, metabolic syndrome, and stressful lifestyle, many individuals suffer from hyperlipidemia, which could mount the risk of cardiovascular diseases [24]. Today used medications to treat dyslipidemia have several adverse effects. For example, statins induced abnormal liver function test, fibrates may present renal insufficiency, and so on [4]. Hence, a new drug with lower adverse effects is well sensed. Esculetin (6,7-dihydroxycumarin) is a kind of pharmaceutical agent which is produced as a lipoxygenase inhibitor derived from coumarin and is found naturally in many herbal plants such as *Cortex fraxini*. esculetin is found to have antineoplastic, anti-inflammatory, anticoagulative, antioxidative, antibacterial, and antidiabetic effects. Esculetin is also found to induce apoptosis in adipocytes, and thus, decrease the adipose mass of the body and decrease the risk of metabolic syndrome and cardiovascular diseases [25-27]. Thus, esculetin could be a suitable choice as a new agent in the treatment of dyslipidemia, even though more researches are mandatory.

In the present animal study, the role of esculetin in the redaction of rabbits' lipid profile was studied according to the molecular pharmaceutical structure of that. Dozens of studies indicated that esculetin might have a significant role in diminishing TG, LDL cholesterol, and total cholesterol. Notwithstanding, several articles express that it does not play a major role in decreasing lipid profile [25]. Satheesh et al. illustrated that coumarin agents such as esculetin have a dominant role in the redaction of TG, LDL cholesterol, and total cholesterol levels [28]; however, Tasdemir E. et al. showed that there is not a powerful correlation between escultin use and a decrease in lipid profile; also Tesdemir E et al. indicated that esculetin might have a marginal role in alleviating hyperlipidemia symptoms [25]. Therefore, several controversies might exsiccate in this condition. Our results support the theory that esculetin has a prominent role as a dyslipidemia treatment. In addition, **figures 3, and 4** illustrate the mean difference of HDL cholesterol, total cholesterol, TG, and LDL cholesterol in our experiment before and after the intervention.
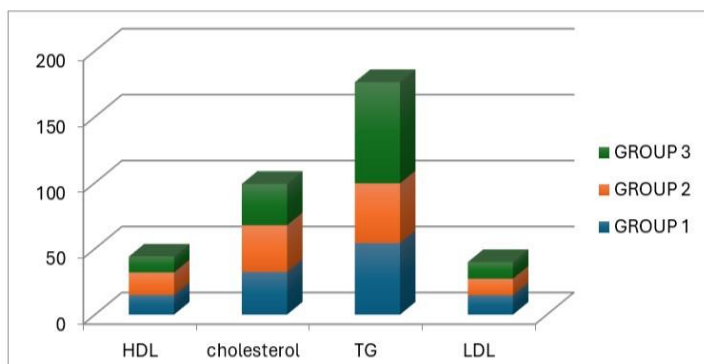

Figure 3: The bar chart depicts the mean of our subject lipid profile before intervention in three separate groups.
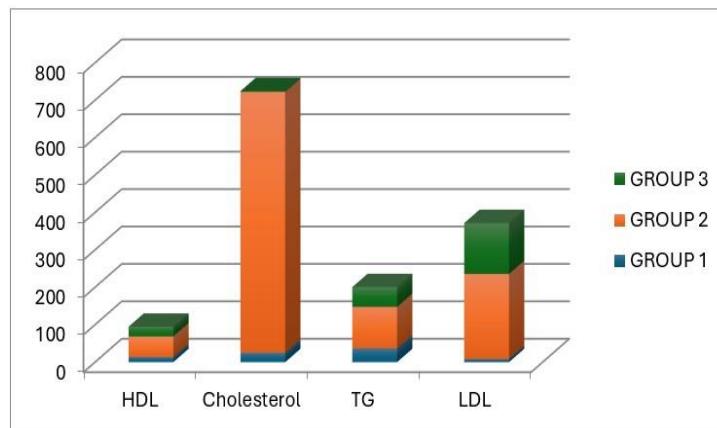

Figure 4: The bar chart gives information about the post-intervention mean of the lipid profile component.

Karthika et al. evaluated the effects of esculetin on lipoperoxides and antioxidants in rats with myocardial infarction induced by Isoproterenol. They found that esculetin would decrease the level of plasma thiobarbituric acid reactive substances (TBARS), plasma hydroperoxides (HP), Heart TBARS, heart HP, and increase the level of Superoxide dismutase, plasma ceruloplasmin, Heart glutathione, as well as Vitamin C and E in the plasma and the heart. However, the levels of the mentioned variables did not differ between control rats and the ones who received esculetin only without Isoproterenol [29]. The findings of Karthika indicated that esculetin could have cardio-protective effects in rats with myocardial infarction but did not have a prophylactic effect. In our study, on the other hand, we found that since esculetin can improve the lipid profile in rabbits, it might have a prophylactic effect against major cardiovascular diseases.

Our animal trial depicts that esculetin significantly reduces the level of TG and Cholesterol; thus, it could help to treat dyslipidemia and diminish the cardiovascular risk of high lipid profile condition. Although numerous studies support our result, Wang et al. illustrated that esculetin is able to reduce the level of LDL cholesterol [24]. In our study, the LDL cholesterol level of the intervention group was reduced, although the result is not significant. Furthermore, numerous studies express that the level of HDL cholesterol was decreased in their studies [24,30,31]; in contrast, Tasdamir E. et al. showed that HDL cholesterol level was increased due to esculetin in their experimental rats. The HDL cholesterol level was mounted in our trial despite it does not have statistically significant meaning. Strongly, further studies with larger sample sizes are necessary to draw a more precise conclusion.

Choi and colleagues also conducted an animal study on diabetic mice with non-alcoholic fatty liver disease, which showed that esculetin could decrease the level of systemic inflammation and reverse the changes seen in the histopathology of the liver during non-alcoholic fatty liver disease [32]. Such findings are in line with our study that esculetin can enhance the condition of patients with dyslipidemia and prevent its negative outcomes.

Conclusions:

In this study, we found that esculetin could prevent weight gain due to high-fat diets; moreover, it was effective in decreasing the total cholesterol and triglyceride levels in New Zealand rabbits. However, it was not able to increase the HDL cholesterol level or decrease the LDL cholesterol level significantly.

Limitations:

It would be better if we could have baseline and post-intervention lab studies such as serum creatinine, liver function tests, and thyroid function tests, among others, to better assess the safety of esculetin in mammalian subjects. Moreover, hepatic and myocardial pathologic specimens would give beneficial information; however, due to the lack of financial resources, it was not possible for us.

Recommendations:

Further studies must be conducted on the safety and efficacy of esculetin in the treatment of dyslipidemia in mammalian subjects so that it could be tested on human subjects later.

Declaration:

Acknowledgments:

Not applicable.

Ethics approval:

This interventional study followed an animal clinical trial protocol and reported in accordance with ARRIVE guidelines. All the procedures were reviewed and approved by the Committee for Animal Experiments at the Jahrom University of Medical Sciences. all experiments were performed in accordance with relevant animal study guidelines and regulations.

Conflict of interests:

The authors declare no conflict of interests.

Consent for publication:

Not applicable

Availability of data and materials:

The datas are also available on request from the corresponding author.

Competing interests:

The authors declare no competing interests.

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References:

1. Pirillo A, Casula M, Olmastroni E, Norata GD, Catapano AL. Global epidemiology of dyslipidaemias. Nature Reviews Cardiology. 2021;18(10):689-700.
2. Karantas DI, Okur EM, Okur, Neslihan, Siafaka IP. Dyslipidemia Management in 2020: An Update on Diagnosis and Therapeutic Perspectives. Endocrine, Metabolic & Immune Disorders - Drug Targets. 2021;21(5):815-34.
3. Zodda D, Giammona R, Schifilliti S. Treatment Strategy for Dyslipidemia in Cardiovascular Disease Prevention: Focus on Old and New Drugs. Pharmacy (Basel). 2018;6(1).
4. Kopin L, Lowenstein CJ. Dyslipidemia. Annals of Internal Medicine. 2017;167(11):ITC81-ITC96.
5. Jang AY, Lim S, Jo S-H, Han SH, Koh KK. New Trends in Dyslipidemia Treatment. Circulation Journal. 2021;85(6):759-68.
6. Zhang L, Xie Q, Li X. Esculetin: A review of its pharmacology and pharmacokinetics. Phytotherapy Research. 2022;36(1):279-98.
7. Edathara PM, Chintalapally S, Makani VKK, Pant C, Yerramsetty S, M DR, et al. Inhibitory role of oleanolic acid and esculetin in HeLa cells involve multiple signaling pathways. Gene. 2021; 771:145370.
8. Zhang G, Xu Y, Zhou HF. Esculetin Inhibits Proliferation, Invasion, and Migration of Laryngeal Cancer In Vitro and In Vivo by Inhibiting Janus Kinas (JAK)-Signal Transducer and Activator of Transcription-3 (STAT3) Activation. Med Sci Monit. 2019;25:7853-63.
9. Singh L, Kaur A, Garg S, Singh AP, Bhatti R. Protective Effect of Esculetin, Natural Coumarin in Mice Model of Fibromyalgia: Targeting Pro-Inflammatory Cytokines and MAO-A. Neurochem Res. 2020;45(10):2364-74.
10. Garg SS, Gupta J, Sahu D, Liu C-J. Pharmacological and Therapeutic Applications of Esculetin. International Journal of Molecular Sciences [Internet]. 2022; 23(20).
11. Kim Y, Lee J. Esculetin Inhibits Adipogenesis and Increases Antioxidant Activity during Adipocyte Differentiation in 3T3-L1 Cells. Prev Nutr Food Sci. 2017;22(2):118-23.
12. Arora R, Sawney S, Saini V, Steffi C, Tiwari M, Saluja D. Esculetin induces antiproliferative and apoptotic response in pancreatic cancer cells by directly binding to KEAP1. Mol Cancer. 2016;15(1):64.
13. Witaicenis A, Luchini AC, Hiruma-Lima CA, Felisbino SL, Justulin LA, Garrido-Mesa N, et al. Mechanism and Effect of Esculetin in an Experimental Animal Model of Inflammatory Bowel Disease. European Journal of Inflammation. 2013;11(2):433-46.
14. Prabakaran D, Ashokkumar N. Protective effect of esculetin on hyperglycemia-mediated oxidative damage in the hepatic and renal tissues of experimental diabetic rats. Biochimie. 2013;95(2):366-73.
15. Pullaiah CP, Nelson VK, Rayapu S, G V NK, Kedam T. Exploring cardioprotective potential of esculetin against isoproterenol induced myocardial toxicity in rats: in vivo and in vitro evidence. BMC Pharmacology and Toxicology. 2021;22(1):43.
16. Zaragozá C, Zaragozá F, Gayo-Abeleira I, Villaescusa L. Antiplatelet Activity of Coumarins: In Vitro Assays on COX-

1. Molecules. 2021;26(10).

17. Neichi T, Koshihara Y, Murota S. Inhibitory effect of esculetin on 5-lipoxygenase and leukotriene biosynthesis. Biochim Biophys Acta. 1983;753(1):130-2.

18. Sekiya K, Okuda H, Arichi S. Selective inhibition of platelet lipoxygenase by esculetin. Biochim Biophys Acta. 1982;713(1):68-72.

19. Jalkanen A, Lassheikki V, Torsti T, Gharib E, Lehtonen M, Juvonen RO. Tissue and interspecies comparison of catechol-O-methyltransferase mediated catalysis of 6-O-methylation of esculetin to scopoletin and its inhibition by entacapone and tolcapone. Xenobiotica. 2021;51(3):268-78.

20. Ozaki Y, Ohashi T, Niwa Y. A comparative study on the effects of inhibitors of the lipoxygenase pathway on neutrophil function. Inhibitory effects on neutrophil function may not be attributed to inhibition of the lipoxygenase pathway. Biochem Pharmacol. 1986;35(20):3481-8.

21. Kotlyarov S. Genetic and Epigenetic Regulation of Lipoxygenase Pathways and Reverse Cholesterol Transport in Atherogenesis. Genes (Basel). 2022;13(8).

22. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Jr., et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation. 1995;92(5):1355-74.

23. Fick TE, Schalm SW. A procedure for arterial blood sampling in the rabbit. Laboratory Animals. 1986;20(2):138-9.

24. Wang Y-H, Liu Y-H, He G-R, Lv Y, Du G-H. Esculin improves dyslipidemia, inflammation and renal damage in streptozotocin-induced diabetic rats. BMC Complementary and Alternative Medicine. 2015;15(1):402.

25. Taşdemir E, Atmaca M, Yıldırım Y, Bilgin HM, Demirtaş B, Obay BD, et al. Influence of coumarin and some coumarin derivatives on serum lipid profiles in carbontetrachloride-exposed rats. Human & Experimental Toxicology. 2016;36(3):295-301.

26. Liang C, Ju W, Pei S, Tang Y, Xiao Y. Pharmacological Activities and Synthesis of Esculetin and Its Derivatives: A Mini-Review. Molecules [Internet]. 2017; 22(3).

27. Tanideh N, Nabavizadeh SS, Ashkani-Esfahani S, Hosseinabadi OK, Ghaemmaghami P, Zare S, et al. Paracrine effect of synovial-derived stem cells on induced knee osteoarthritis in rats. Shiraz E-Medical Journal. 2021;22(6).

28. Dharmarajan SK, Arumugam KM. Comparative evaluation of flavone from Mucuna pruriens and coumarin from Ionidium suffruticosum for hypolipidemic activity in rats fed with high Fat diet. Lipids in Health and Disease. 2012;11(1):126.

29. Karthika P, Rajadurai M, Ganapathy P, Kanchana G, editors. Preventive effect of esculetin on lipid peroxides and antioxidants in isoproterenol-induced myocardial infarction in Wistar rats2016.

30. Al-Said MS, Mothana RA, Al-Sohaibani MO, Rafatullah S. Ameliorative Effect of Grewia tenax (Forssk) Fiori Fruit Extract on CCl4–Induced Oxidative Stress and Hepatotoxicity in Rats. Journal of Food Science. 2011;76(9):T200-T6.

31. Nabavizadeh SS, Talaei-Khozani T, Zarei M, Zare S, Hosseinabadi OK, Tanideh N, et al. Attenuation of osteoarthritis progression through intra-articular injection of a combination of synovial membrane-derived MSCs (SMMSCs), platelet-rich plasma (PRP) and conditioned medium (secretome). Journal of Orthopaedic Surgery and Research. 2022;17(1):1-12.

32. Choi R-Y, Ham JR, Lee M-K. Esculetin prevents non-alcoholic fatty liver in diabetic mice fed high-fat diet. Chemico-Biological Interactions. 2016;260:13-21.