International Journal of Disaster Recovery and Business Continuity
Vol.12, No. 1, (2021), pp. 01–08

Glycyrrhizic Acid And Its Derivatives As The Carriers For The Poorly Soluble Flavonoids

Quzijon BARATOV[1,2], Mukhammadjon MUSTAFAKULOV[2], Alimjon MATCHANOV[1], Natalia VYPOVA[1], Rana YAKUBOVA[1], Nigora TAGAYALIEVA[1,3]

[1]A. S. Sadykov Institute of Bioorganic Chemistry, AS RUz,
[2]Institute of Biophysics and Biochemistry under Mirzo Ulugbek
National University of Uzbekistan, Tashkent, Uzbekistan
[3]tnigora@mail.ru

Abstract

Rutin is among the most explored and widely used flavonoids, but poor solubility limiting its usage triggers search for methods to improve its bioavailability. We offered to use glycyrrhizic acid and its derivatives as the carriers for rutin in supramolecular complexes.

Aim *The work was initiated to comparatively study effects of two low dose rutin complexes, such as the one with glycyrrhizic acid (GA/rutin) and the one with monoammonium salt of glycyrrhisic acid (GAMS/rutin) in rats with the induced hyperlipidemia.*

Methods *Rutin, as well as the GA/rutin and the GAMS/rutin complexes were administered to the animals per orally at the dose of 40 mg/kg for 10 days after induction of hyperlipidemia by Tween-80 (polysorbate 80).*

Results *In the rats with the induced hyperlipidemia, the elevated total cholesterol ($p<0.00001$), triglycerides (TG) ($p<0.00001$), low density lipoproteins (LDL) ($p<0.01$), and malondialdehyde (MDA) ($p<0.00001$), as well as a reduction in blood catalase activity ($p<0.05$) and high density lipoproteins (HDL) was registered. Rutin was found to reduce total cholesterol ($p<0.01$) and to increase catalase activity ($p<0.05$). The GAMS/rutin complex reduced total cholesterol ($p<0.01$), TG ($p<0.001$) and MDA ($p<0.05$), increasing HDL ($p<0.05$). The GA/rutin complex reduced total cholesterol ($p<0.01$), TG ($p<0.0001$), LDL ($p<0.05$) and MDA concentrations ($p<0.01$), increasing HDL ($p<0.01$) and catalase activity ($p<0.05$). The GA/rutin complex was found more efficient than rutin and the GAMS/rutin complex for some parameters under study.*

Conclusion *as the result, the highly active supramolecular GA/rutin complex with GA and rutin ratio 4:1 called Biorutin has been chosen for further studies.*

Keywords: *rutin, glycyrrhizic acid, supramolecular complex, hyperlipidemia, antioxidant*

Due to efficient therapeutic properties undoubtedly proved, medicinal plants have gained worldwide currency in the public health care systems [1]. With considerable diversity of bioactive natural compounds, phenolic compounds, including flavonoids, are the well-represented ones [2]. Rutin and quercetin, flavonoids with the established antioxidant, anti-inflammatory, cyto- and vasoprotective, anti-tumor, neuro- and cardioprotective, antimicrobial and antibacterial activities, are among the most explored and widely used [3]. But for all the significant biological activity the flavonoids demonstrate in various systems *in vitro*, their low bioavailability limits their biological effects *in vivo*. Appropriate delivery systems for rutin or generation of its highly soluble derivatives may improve its bioavailability, thus increasing its significance and future look in prevention and/or treatment of various chronic human diseases. With a view to that, a variety of methods enhancing the solubility of rutin both in water and in a lipid fraction was set forward. These include generation of (i) nanoformulations in the form of nanoscale capsules or emulsions, including the gelled ones, (ii) rutin complexes with cyclodextrin or phospholipids, (iii) various types of rutin derivatives, to name the hydroxyethylized ones (Venoruton, a vasoprotective), carboxyl and sulfonate derivatives providing 100-fold enhancement of rutin solubility, (iv) the synthesis of oligomers providing 4,200-fold enhancement of rutin solubility, etc. [4,5].

More specifically, a group of Italian researchers microencapsulated rutin in a chitosan matrix using the spray-drying technique. The microparticles had a smooth surface making possible a

ISSN: 2005-4289IJDRBC

controlled release of the compound. Anti-inflammatory activity of the rutin-loaded microspheres was confirmed improved both *in vivo* and *in vitro* [6]. Babazadeh et al. reported on production of rutin encapsulated with phosphatidylcholine forming a PC-rutin complex called nanophytosome to enhance rutin hydro solubility and bioavailability [7]. Rutin can be used in combination with ionic liquids to improve its delivery, bioavailability and efficacy, as well [8].

Earlier on, we reported on generation of supramolecular rutin complexes with glycyrrhizic acid and monoammonium salt as carriers for the drug and comparative characterization of their capillary protective and antioxidant activities in physiological standard *in vivo* [9].

The work was initiated to continue studying effects of two low dose rutin complexes by their lipid lowering and antioxidant activities in mice with the induced hyperlipidemia *in vivo,* as rutin demonstrated the effects both at its administration [10] and after the onset of hyperlipidemia [11].

MATERIALS AND METHODS

Lipid lowering activity of products under study in the model of induced hyperlipidemia in vivo

Fifty outbred white male rats with basal body weight 400±50g kept at the animal facility of laboratory of pharmacology, Institute of Bioorganic Chemistry, Uzbekistan Academy of Sciences, were used in the experiments. The animals were kept under standard conditions in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes [12].

The animals were randomly divided into five groups with 10 rats in each. Thus, intact healthy rats were included into the I group, the II group was the control one, animals in the III group received rutin (comparison group) and those in the IV and V groups were administered with the GA/rutin complex and the GAMS/rutin complex, respectively (experimental groups).

To induce hyperlipidemia, alimentary cholesterol was additionally administered to animals of II-V groups for a month followed by a 10-day intraperitoneal administration of tween-80, a detergent, at the dose of 200 mg/kg [13]. After hyperlipidemia onset, animals of the III group received 40 mg/kg rutin solution per orally once a day for 10 days, those from the IV group received 40 mg/kg of glycyrrhizic acid (GA)/rutin complex (4:1) and those from the V group were administered with 40 mg/kg of glycyrrhizic acid monoammonium salt (GAMS)/rutin complex (4:1). The controls received equivalent volume of water per orally.

On the 10^{th} day the animals were removed from the experiment under ether anesthesia to measure:

(i) concentrations of malondialdehyde (MDA), secondary product of lipid peroxidation, a marker for oxidative stress in blood by reaction of formation of the stained trimethyl complex with 2-thiobarbituric acid at high temperature and acidity spectrophotometrically at maximum absorption spectrum of 532 nm [13],

(ii) catalase activity in blood by the presence of ammonium molybdate spectrophotometrically at 410 nm [14],

(iii) concentrations of total cholesterol, triglycerides (TG), low density lipoproteins (LDL), high density lipoproteins (HDL) using biochemical analyzer with a test system (Cypress Diagnostics, Belgium), as well as the atherogenic index (AI) calculated using the following formula: AI=(total cholesterol – LDL)/HDL.

The data were statistically processed with calculation of arithmetic mean (M), average error of arithmetic mean (m), Student's criterion (t) and error probability (p). The changes were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Lipid lowering activity of rutin and its complexes

The findings from the study on lipid lowering activity of rutin and its complexes in rats with the induced hyperlipidemia *in vivo* can be seen in Table 1. As it can be seen, in the controls the onset of hyperlipidemia was marked by the significant increase in total cholesterol from 1.39±0.05 to

ISSN: 2005-4289IJDRBC

2.03±0.05 mmol/l ($p<0.00001$), in triglycerides (TG) from 0.91±0.01 to 1.44±0.07 mmol/l ($p<0.000001$) and in low density lipoproteins (LDL) from 0.88±0.03 to 1.52±0.21 mmol/l ($p<0.01$). High density lipoproteins (HDL) were found to decline from 0.35±0.05 to 0.22±0.038 mmol/l, though not reaching statistical significance.

Rutin and its complexes administered at the dose of 40 mg/kg were found to reduce total cholesterol significantly; the GA/rutin complex reduced total cholesterol most efficiently (to 1.58±0.04 mmol/l, $p<0.0001$), while rutin and the GAMS/rutin complex demonstrated lower effect in reduction of total cholesterol (to 1.70±0.04 mmol/l, $p<0.01$ and 1.73±0.01 mmol/l, p<0,001, respectively). Nonetheless, total cholesterol concentrations were significantly higher than those in the intact animals ($p<0.01$ for all formulations). At the same time, the GA/rutin complex reduced total cholesterol more efficiently than rutin ($p<0.05$) and the GAMS/rutin complex ($p<0.005$).

The rutin-based complexes, but not rutin as it is, significantly reduced concentrations of triglycerides. The GA/rutin complex was found to reduce TG to 1.07±0.02 mmol/l ($p<0.0001$), while the GAMS/rutin complex reduced TG to 1.14±0.03 mmol/l ($p<0.001$), but these values are significantly higher than the physiological standard ($p<0.000001$).

Table 1. Lipid lowering activity of rutin and its complexes in rats with the induced hyperlipidemia *in vivo*, M±m, n=10

	Physiological standard (n=10)	**Control (n=10)**	**Rutin, 40 mg/kg (n=10)**	**GA/Rutin, 40 mg/kg (n=10)**	**GAMS/Rutin, 40 mg/kg (n=10)**
Total cholesterol, mmol/l	1.39±0.05	2.03±0.05 $p_1<0.00001$	1.70±0.04 $p_1<0.01$ $p_2<0.01$ $p_3<0.05$	1.58±0.04 $p_1<0.01$ $p_2<0.0001$	1.73±001 $p_1<0.01$ $p_2<0.01$ $p_3<0.005$
TG, mmol/l	0.91±0.01	1.44±0.07 $p_1<0.000001$	1.22±0.16	1.07±0.02 $p_1<0.000001$ $p_2<0.0001$	1.14±0.03 $p_1<0.000001$ $p_2<0.001$
HDL, mmol/l	0.35±0.05	0.22±0.038	0.36±0.09	0.38±0.03 $p_2<0.01$	0.34±0.02 $p_2<0.05$
LDL, mmol/l	0.88±0.03	1.52±0.21 $p_1<0.01$	1.11±0.04 $p_1<0.001$ $p_3<0.01$	0.98±0.02 $p_1<0.05$ $p_2<0.05$	1.15±0.04 $p_1<0.0001$ $p_3<0.01$
AI	2.97	8.23	3.72	3.16	4.09

Note: p_1 - significance of differences as compared to those in intact animals, p_2 - significance of differences as compared to those in the control, p_3 - significance of differences with parameters demonstrated by the GA/rutin complex

As to elevated LDL concentrations, they were modified by all the formulations under study, but only the GA/rutin complex reduced them significantly with reference to the control values (to 0.98±0.02 mmol/l, ($p<0.05$), and even to those observed in animals administered with rutin (1.11±0.04 mmol/l, $p<0.01$) and with the GAMS/rutin complex (1.15±0.04 mmol/l, $p<0.001$). In each case, the treatment caused no reduction of LDL concentrations comparable to those of physiological standard; the differences preserved their statistical significance.

It is far from the case with the HDL concentrations. All formulations under study caused elevation of the concentrations up to the physiological standard; parameters demonstrated by both rutin-based complexes turned out significantly higher than the control ones. They were 0.38±0.03 mmol/l ($p_2<0.01$) and 0.34±0.02 mmol/l ($p_2<0.05$) for the GA/rutin and the GAMS/rutin complex, respectively.

As the result, atherogenic index (AI) in the controls was nearly 3 times higher than the one in the intact animals (8.23 versus 2.97, respectively), while in animals receiving the GA/rutin complex the parameter was the lowest one (3.16) and comparable with the one typical for physiological standard.

ISSN: 2005-4289IJDRBC

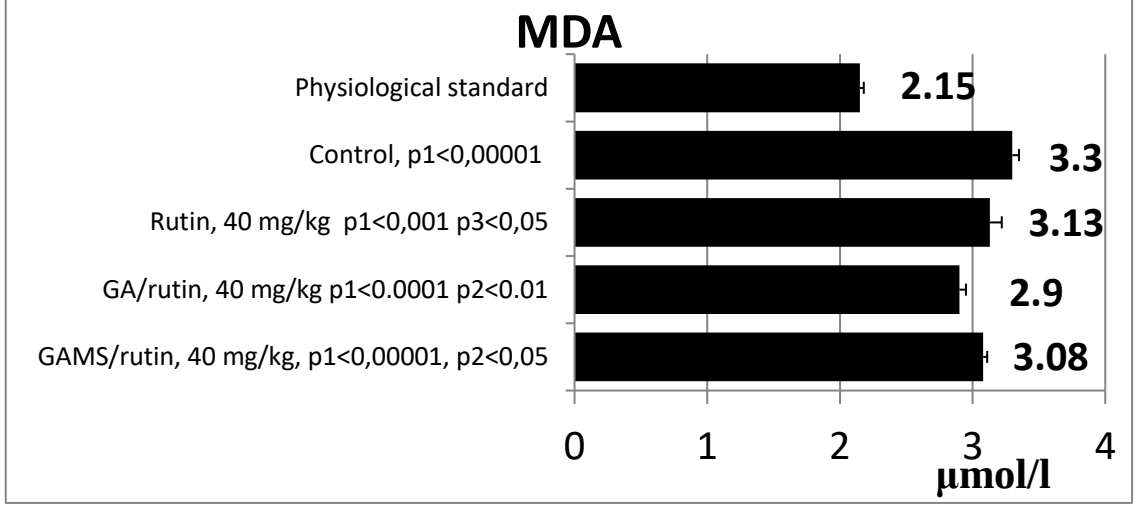
Our findings are consistent with those of other researchers. Thus, Al-Rejaie et al. demonstrated that treatment of rats with 0.2% rutin caused a reduction in total cholesterol ($p<0.05$), TG and LDL (both $p<0.05$), and an elevation in HDL [16]. da Silva et al. reported that rutin significantly reduced the concentrations of total cholesterol, TG, LDL and very low density lipoproteins, not lowering HDL [17]. In the hypercholesterolemic golden Syrian hamsters with body weight of 100 ±20g, rutin isolated from *Dimorphandra mollis*, a common plant in Brazil, and added in 0.8% by mass was shown to cause a reduction in TG, changing neither total cholesterol nor HDL [18].

The inhibition of cholesterol in primary rat hepatocytes *in vitro* by quercetin, a key metabolite of rutin, can be considered as the underlying mechanism for the lipid lowering effect of rutin [19].

The inhibition of destruction of lipoproteins by direct extinguishing of free radicals of various geneses by molecules of antioxidant flavonoids could be another mechanism determining protective role of rutin upon atherosclerotic changes [20].

Antioxidant activity of rutin and its complexes

Subsequent to Xepapadaki and co-author [21], we studied the parameters of oxidative stress, a component of pathogenesis of atherosclerosis, among other things associated with lipid metabolism disorders. Hyperlipidemia with high AI induced in the control animals caused reduction in the antioxidant activity; MDA, a secondary product of lipid peroxidation, was found to significantly increase from 2.15±0.03 to 3.30±0.05 μmol/l ($p_1<0.00001$) (Fig.1). The treatment with the GA/rutin complex resulted in significant reduction in MDA concentrations to 2.90±0.05 μmol/l ($p_2<0.01$ with reference to control) and to 3.08 ±0.03 μmol/l ($p_2<0.05$) in case of treatment with the GAMS/rutin complex. But in both cases, the parameters were above the standard ($p_1<0.0001$). Rutin administered at the dose of 40mg/kg produced no significant effect on the MDA concentrations, its antioxidative activity turned out lower than the one of the GA/rutin complex ($p_3<0.05$).



A

B

ISSN: 2005-4289IJDRBC

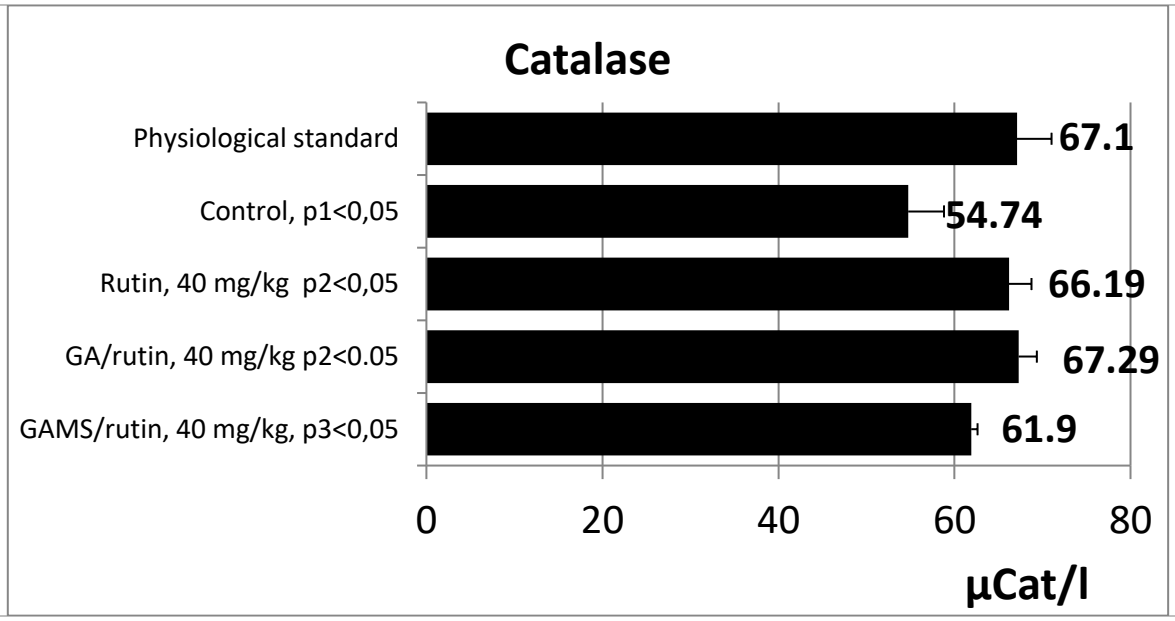


Figure 1. Antioxidative activity of rutin and its complexes in hyperlipidemic rats *in vivo.*
A. MDA concentrations in blood.
B. Blood catalase activity
(Note: p_1 - significance of differences as compared to those in intact animals, p_2 - significance of differences as compared to those in the control, p_3 - significance of differences with parameters demonstrated by the GA/rutin complex)

A decline in antioxidative activity of an organism is indicative of the reduction in activity of antioxidant enzymes, to name superoxide dismutase, ceruloplasmin and blood catalase widely used as clinical markers for diagnosis [22]. Our study demonstrated a significant decline in blood catalase activity from 67.1±3.93 to 54.74±4.07 µCat/l ($p<0.05$) in animals with the induced hyperlipidemia. Treatment with rutin and GA/rutin produced significant ($p<0.05$) therapeutic effect on blood catalase activity elevating the parameter to 66.19±2.56 and 67.29±2.07µCat/l, respectively. At the same time, the GAMS/rutin complex caused no significant changes in the activity of the enzyme, demonstrating significant differences with the parameters for the GA/rutin (61.9±0.72 µCat/l).

According to Al-Rejaie et al. [16], regulation of the expression levels of some genes involved in the oxidative stress pathway is the mechanism of enhancing the antioxidant effect of rutin. Addition of 1% cholesterol and 0.5% cholic acid to rat chow to induce alimentary hyperlipidemia was shown to trigger changes in expression levels of the genes involved in the oxidative stress pathway causing DNA damage and hepatotoxicity. 0.2% rutin in rat chow caused a decrease in the genes expression levels of glutathione peroxidase (GPx) and glutathione reductase (GR), and an increase in those of glutathione S transferase α (GSTα), sulfiredoxin-1(Srx1), glutamate-cysteine ligase (GCL) and paraoxonase-1(PON-1), that is, enhancing the antioxidant effect.

In our study, rutin at the dose of 40 mg/kg was shown to significantly reduce total cholesterol and elevate blood catalase activity; there were no significant effects on the other parameters under study. The GA/rutin and GAMS/rutin complexes administered at the same dose turned out even more efficient. Most likely, it is improvement of rutin bioavailability imparted by both carriers to the flavonoid that underlie the elevation in question. In addition, contrary to other carriers of rutin [5], GA in a complex plays a dual role of an active ingredient with intrinsic high potencies *per se* and of a carrier for another agent [23]. In addition, a synergism of GA and rutin should be taken into account [24] considering the weight percentage of rutin in the complexes under study (near 16%).

As Tykarska и Gdaniec demonstrated, chemical differences between GA and its mono- and dibasic salts produce little if any effect on the supramolecular organization of neutral and ionic forms of the acid [25]. But our findings demonstrated that the structure of a carrier indeed had an impact on

ISSN: 2005-4289IJDRBC

the biological effects of the complexes under study. Specifically, there were significant differences in the effects produced by two GA complexes on total cholesterol, LDL and blood catalase activity in our model of the induced hyperlipidemia. Earlier on, we demonstrated significant decline in the capillary permeability in mice with the induced aseptic inflammation after single dosing of the GA/rutin and GAMS/rutin complexes versus the controls; the capillary protective effect of the former was found more significant than of the latter one ($p<0.01$). The GA/rutin complex was found more efficient in assessment of antioxidant activity (as per MDA level) in the physiological standard condition ($p<0.00005$) [9].

In the recent review where the therapeutic benefits of rutin and its nanoformulations were summarized it was pointed out that as antioxidant agents rutin neutralizes free radicals and chelates transition metals. The antioxidant capacity and the inhibitory effects of rutin on the lipid peroxidation was depended on its concentration. Effect of nanosizing on the pharmacological effect is an increase in the antioxidant activity and an enhancing of the oral bioavailability [5]. In our case, the use of GA as a carrier for rutin to increase solubility and, accordingly, bioavailability also leads to an increase in its hypolipidemic properties and antioxidant activity.

CONCLUSIONS

In an experimental model of induced hyperlipidemia, supramolcular complexes of rutin with GA and GAMS showed greater antioxidant and hypolipidemic activity than rutin itself. In a comparative aspect, GA turned out to be a more effective carrier, despite the same supramolecular organization of the complexes.

As the result, the highly active supramolecular GA/rutin complex with GA and rutin ratio 4:1 called Biorutin has been chosen for further studies as the one with significant lipid lowering and antioxidant properties. The further studies should be aimed at the choice of its optimal dose of administration.

ACKNOWLEDGEMENTS

We are grateful to the staff members of A. S. Sadykov Institute of Bioorganic Chemistry, AS RUz and the Institute of Biophysics and Biochemistry under Mirzo Ulugbek National University of Uzbekistan for the cooperation and support in our research.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Shukla A. C. The Herbal Drugs //Advances in Pharmaceutical Biotechnology. – Springer, Singapore, 2020. – P. 69-75.
2. Jucá M. M., Cysne Filho F. M. S., de Almeida J. C., Mesquita D. D. S., Barriga J. R. D. M., Dias K. C. F., Barbosa T.M., Vasconcelos L.C., Leal L.K.A.M., Ribeiro J.E.,Vasconcelos S. M. Flavonoids: biological activities and therapeutic potential //Natural product research. – 2020. – Vol. 34. – №. 5. – P. 692-705.
3. Luca S. V., Macovei I., Bujor A., Miron A., Skalicka-Woźniak K., Aprotosoaie A. C., Trifan A. Bioactivity of dietary polyphenols: The role of metabolites //Critical Reviews in Food Science and Nutrition. – 2020. – Vol. 60. – №. 4. – P. 626-659.
4. Gullón B., Lú-Chau T. A., Moreira M. T., Lema J. M., Eibes G. Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability //Trends in food science & technology. – 2017. – Vol. 67. – P. 220-235.
5. Negahdari R., Bohlouli S., Sharifi S., Maleki Dizaj, S., Rahbar Saadat, Y., Khezri, K., Jafari S., Ahmadian E., Negar G. Jahandizi N.G., Raeesi, S. (2020). Therapeutic benefits of rutin and its nanoformulations//Phytotherapy Research. – 2020. – p.1–20.
6. Cosco D., Failla P., Costa N., Pullano S., Fiorillo A., Mollace V., Fresta M, Paolino D. Rutin-loaded chitosan microspheres: Characterization and evaluation of the anti-inflammatory activity //Carbohydrate polymers. – 2016. – Vol. 152. – P. 583-591.
7. Babazadeh A., Ghanbarzadeh B., Hamishehkar H. Phosphatidylcholine-rutin complex as a potential nanocarrier for food applications //Journal of Functional Foods. – 2017. – Vol. 33. – P. 134-141.

ISSN: 2005-4289IJDRBC

8. Caparica R., Júlio A., Araújo M. E. M., Baby A. R., Fonte P., Costa J. G., Santos de Almeida T. Anticancer Activity of Rutin and Its Combination with Ionic Liquids on Renal Cells //Biomolecules. – 2020. – Vol. 10. – №. 2. – P. 233.
9. Baratov K.R.U., Makhmudov L.U., Matchanov U.D., Tagayalieva N.A. Comparative biological activity of supramolecular complexes with rutin. // Universum: chemistry and biology: academic journal – № 8(74). Part 1. – 15-18 (in Russian).
10. Manzoni A. G., Passos D. F., da Silva J. L., Bernardes V. M., Bremm J. M., Jantsch M. H., de Oliveira J. S., Mann T. R., de Andrade C.M., Leal D. B. Rutin and curcumin reduce inflammation, triglyceride levels and ADA activity in serum and immune cells in a model of hyperlipidemia //Blood Cells, Molecules, and Diseases. – 2019. – V. 76. – 13-21.
11. Sattanathan K., Dhanapal C.K., Manavalan R. LDL lowering properties of rutin in diabetic patients. Int J Pharm Bio Sci 2010;4:467-473.
12. European Directive 2010/63/EU on the protection of animals used for scientific purposes. September 22, 2010. Official Journal of the European Union, L 276/33- L276/79
13. Vaskanyan V.L., Vasilenko Yu.K., Ponomaryov V.D. Lipid lowering properties of saparal and oleanolic acid // Pharmaceutical chemistry journal — 1983. — No2. - 49–52 (in Russian)
14. Stalnaya I.D., Garishivili T.G. Method to determine malondialdehyde by thiobarbituric acid. // Up-to-date methods in biochemistry. / Edited by B.N. Orekhovich. – M.: Meditsina, 1977- 66-68 (in Russian).
15. Korolyuk M.A., Ivanova L.I., Mayorova I.G., Tokarev B.E. Methods to determine catalase activity // Moscow, Meditsina, 1988, -16-19 (in Russian).
16. Al-Rejaie, S.S., Aleisa A.M., Sayed-Ahmed M.M., AL-Shabanah O.A., Abuohashish H. M., Ahmed M. M., Al-Hosaini K.A., Hafez M. M. Protective effect of rutin on the antioxidant genes expression in hypercholestrolemic male Westar rat. //BMC complementary and alternative medicine. – 2013. – 13. – 1. – 1-9.
17. da Silva R.R., de Oliveira T.T., Nagem T.J., Pinto A.S., Albino L.F., de Almeida M.R., de Moraes G.H., Pinto J.G. Efeito hipolipidêmico dos flavonóides naringina e rutina [Hypocholesterolemic effect of naringin and rutin flavonoids] //Archivos latinoamericanos de nutricion. – 2001. – 51. – 3. – 258-264. (in Portuguese).
18. Kanashiro A., Andrade D. C., Kabeya L. M., Turato W. M., Faccioli L. H., Uyemura S. A., Lucisano-Valim Y. M. Modulatory effects of rutin on biochemical and hematological parameters in hypercholesterolemic Golden Syrian hamsters//Anais da Academia Brasileira de Ciências. – 2009. – 81. – 1. – 67-72.
19. Glässer G., Graefe E. U., Struck F., Veit M., Gebhardt R. Comparison of antioxidative capacities and inhibitory effects on cholesterol biosynthesis of quercetin and potential metabolites. //Phytomedicine. – 2002. – 9. – 1. – 33-40.
20. Nijveldt R.J., Van Nood E., Van Hoorn D.E., Boelens P.G., Van Norren K., Van Leeuwen P.A. Flavonoids: a review of probable mechanisms of action and potential applications //The American journal of clinical nutrition. – 2001. – Т. 74. – №. 4. – С. 418-425..
21. Xepapadaki E., Zvintzou E., Kalogeropoulou C., Filou S., Kypreos K. E. The Antioxidant Function of HDL in Atherosclerosis //Angiology. – 2020. – 71. – 2. – 112-121.
22. Poznyak A.V., Grechko A.V., Orekhova V.A., Chegodaev Y.S., Wu W. K., Orekhov A. N. (2020). Oxidative Stress and Antioxidants in Atherosclerosis Development and Treatment //Biology. – 2020. – Т. 9. – №. 3. – С. 60.
23. Selyutina O.Y., Polyakov N.E. Glycyrrhizic acid as a multifunctional drug carrier - From physicochemical properties to biomedical applications: A modern insight on the ancient drug // Int J Pharm. – 2019. - 559. – 271-279.
24. Maione F., Minosi P., Di Giannuario A., Raucci F., Chini M.G., De Vita S., Bifulco G., Mascolo N., Pieretti S. Long-Lasting Anti-Inflammatory and Antinociceptive Effects of Acute Ammonium Glycyrrhizinate Administration: Pharmacological, Biochemical and Docking Studies // Molecules, 2019, 24(13), 2453-2458.
25. Tykarska E., Gdaniec M. Toward Better Understanding of Isomorphism of Glycyrrhizic Acid and Its Mono-and Dibasic Salts //Crystal growth & design. – 2013. – 13. – 3. – 1301-1308.

ISSN: 2005-4289IJDRBC

ISSN: 2005-4289IJDRBC