Biomedical & Pharmacology Journal, March 2023. Vol. **16**(1), p. 289-293

Physiological Scrutiny to Appraise a Flavonol Versus Statins

Wissam Sajid Hashim[1]*, Youssef Shakuri Yasin[2], Azal Hamoody Jumaa[3], Marwan I. Al-Zuhairi[3] and Ahmed Hazem Abdulkareem[3]

[1]Department of Physiology and medical Physics, College of Medicine, Al-Muthanna University, Iraq.
[2]Department of Nursing, Bilad Alrafidain University College, Diyala, 32001, Iraq.
[3]Faculty of Pharmacy, Bilad Alrafidain University College, Diyala, 32001, Iraq.
*Corresponding Author E-mail: dr.w80@mu.edu.iq

https://dx.doi.org/10.13005/bpj/2610

(Received: 21 December 2022; accepted: 24 January 2023)

Because of the vast use of statins to control and treat hyperlipidemia, this study was set to compare the most common statins Atorvastatin and Simvastatin with the flavonol Kaempferol considering the unwilled collateral effects of them. Sixty adult albino male rats were allocated into five groups of twelve members to each. It is obvious based on the results that Atorvastatin could cause significant declination in the hemoglobin, hematocrit, platelets and leukocytes while simvastatin could cause a significant declination in leukocytes and platelets, on the other side; the kaempferol could not affect these values comparing with the control group. The lipid profile and the hepatic enzymes like Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were significantly disturbed too in all groups and it was very clear that serum aminotransferases and alkaline phosphatase were significantly elevated in all groups except the Kaempferol comparing with control group at (P=0.05). It is very obvious that kaempferol could ameliorate the lipid profile the antioxidant enzymes and the blood values in a manner which is better than those of statins.

Keywords: Atorvastatin; Antioxidants; Flavonol; Kaempferol; Simvastatin; Statins.

Statins are a vast family of medicaments which are used worldwide as remedies for the elevated levels of blood lipids or the functional disturbances of lipoproteins metabolism.[1] Simvastatin and atorvastatin are members of this family.[2] It is true that statins could lower blood lipids but at the same time they could cause significant disturbances in other body systems, for instance they cause a declination in the hemoglobin, platelets, erythrocytes and leukocytes with marked elevations of hepatic enzymes.[3,4] Hence, atorvastatin and simvastatin are the subject of this study. On other side, the flavonol; kaempferol which is naturally presents in vegetables and fruits particularly in grape,[5] is the subject of this study to be compared with statins. Kaempferol was documented to be effective against many diseases with strong antioxidant features. It can be isolated from tea, broccoli, witch-hazel, propslis, grapefruit, and other plant sources. The pharmacological properties of kaempferol exhibit antioxidant, anti-inflammatory, and anticancer activity. Kaempferol also provides benefits for the treatment of atherosclerosis..[6-8]

MATERIALS AND METHODS

Animals of the experiment

Sixty adult male albino rats of 250-280 grams weights were adopted. The experiment has

continued for two months after passing the period of acclimatization of two months with a strict rules considering the optimal conditions, the standard diet and the special fat diet. The high fat diet was introduced to the concerned groups to induce hyperlipidemia.

Protocol of experiment

1. Control group (C group): Twelve male rats were maintained on a standard diet.[9]
2. Cholesterol group (CHO group): Twelve male rats were maintained on high fat diet-HFD which consists of (lipids 41.5%, carbohydrates 40.2%, and proteins 18.3%).[10]
3. Atorvastatin group (ATST group): Twelve male rats were maintained on HFD and they were dosed once a day orally with atorvastatin 20 mg/day by the use of oral gavage.
4. Simvastatin group (SVST group): Twelve male rats were maintained on HFD and they were dosed once a day orally with simvastatin 17 mg/day by the use of oral gavage. The doses of atorvastatin and simvastatin were depended based upon LD50 mentioned by.[11,12]
5. Kaempferol group (KMP group): Twelve male rats were maintained on HFD and they were injected once a day intraperitoneally with kaempferol 2.5 mg/day. The dose of kaempferol was depended upon a previous study.[13]

Parameters of the study

Dongi (Dongi 120 Italia) device was used to obtain the required test exploiting the sera gained from the animals of this study.

Statistical Analysis

ANOVA one way test was depended using SPSS program version 21 to find out the least significant differences among groups.

Ethical approval

This study was approved by the Ethics Committee of the Scientific Research, College of Medicine, Al-Muthanna University, Iraq (approval no. 415/24.03.2022).

RESULTS

The erythrocytes count or the red blood cells count R.B.C. was clearly affected and significantly elevated in the cholesterol or CHO group while those values of the atorvastatin ATST, simvastatin SVST and kaempferol KMP groups were not affected comparing them with the control

Table 1. Comparison among kaempferol and statins effects on blood parameters of rats

Groups	R.B.C× 10^6 cells/µl	HBg/dl	HCT%	W.B.C.× 10^3 cells/µl	PLT× 10^3 plt/µl
C	6.3 ± 0.53 ^{b}	11.4 ± 0.2 ^{b}	42.7 ± 1.11 ^{b}	8.3 ± 0.11 ^{a}	541.2 ± 33.66 ^{a}
CHO	8.3 ± 0.41 ^{a}	13.3 ± 0.34 ^{a}	51.2 ± 2.22 ^{a}	2.5 ± 0.23 ^{b}	433.2 ± 20.7 ^{b}
ATST	6.5 ± 0.44 ^{b}	10.3 ± 1.08 ^{c}	36.4 ± 2.12 ^{c}	3.1 ± 0.11 ^{b}	276.8 ± 8.77 ^{c}
SVST	6.7 ± 0.22 ^{b}	12 ± 0.3 ^{b}	40.3 ± 1.33 ^{b}	2.7 ± 0.15 ^{b}	311.3 ± 11.55 ^{c}
KMP	6.7 ± 0.21 ^{b}	12.1 ± 0.22 ^{b}	41.2 ± 4.14 ^{b}	8.4 ± 0.8 ^{a}	471.8 ± 42.11 ^{b}
LSD	1.6	1.1	6.3	5.2	69.4

Groups: C=Control, CHO=Cholesterol, ATST= Atorvastatin, SVST= Simvastatin, KMP= Kaempferol. LSD= Least Significant Difference. Similar small letters on number; a, b, c refer to presence of significance or not among groups.

Table 2. Comparison among kaempferol and statins effects on differential leukocytes count of rats

Groups	Neutrophil%	Lymphocyte%	Monocyte%	Eosinophil%	Basophil%
C	60.3 ± 1.01 ^{c}	22.8 ± 1.3 ^{b}	11 ± 1.2 ^{a}	0.4 ± 0.5 ^{d}	1 ± 0.1 ^{c}
CHO	72.2 ± 1.3 ^{a}	17.5 ± 1.2 ^{c}	6.7 ± 1.3 ^{b}	1 ± 0.1 ^{a}	1 ± 0.1 ^{c}
ATST	65.4 ± 1.11 ^{b}	20.6 ± 1.03 ^{b}	7.6 ± 1.4 ^{b}	0.2 ± 0.4 ^{c}	0.4 ± 0.5 ^{d}
SVST	41 ± 1.03 ^{d}	46.6 ± 2.6 ^{a}	6.4 ± 1.1 ^{b}	0.2 ± 0.4 ^{c}	1.6 ± 0.5 ^{a}
KMP	56.2 ±2.01 ^{c}	22.2 ± 1.02 ^{b}	12.3 ± 1.6 ^{a}	0.6 ± 0.5 ^{b}	1.2 ± 0.4 ^{b}
LSD	5.1	3.1	3.4	0.2	0.2

Groups: C=Control, CHO=Cholesterol, ATST= Atorvastatin, SVST= Simvastatin, KMP= Kaempferol. LSD= Least Significant Difference. Similar small letters on number; a, b, c refer to presence of significance or not among groups.

group. The hemoglobin HB concentration and the hematocrit HCT were significantly elevated in the CHO group and significantly declined in the ATST group comparing with the control and the other groups. The leukocytes count or the total white blood cells count W.B.C. and the platelets count PLT were significantly declined in all the groups comparing with the control group at (Pd"0.05); table 1. Considering the differential leukocytes count, the neutrophils were significantly elevated in the CHO and ATST groups with significant declination in the SVST group comparing with the control group. The lymphocytes were significantly declined in the CHO group with significant elevation in the SVST group comparing with the control group. The monocytes significantly declined in all treatment groups accept the KMP group comparing with the control. The eosinophils were significantly declined in the ATST and SVST groups with significant elevation in the CHO and KMP groups comparing with the control group. The basophils were significantly elevated in the KMP and SVST groups with significant declination in the ATST group comparing with the control group at (Pd"0.05); table 2. The lipid profile of all the treatment groups was also affected as it is seen in table 3, where the total serum cholesterol TC was significantly elevated in the CHO group and it was significantly less than the CHO group considering the ATST and SVST groups but it was also significantly higher than the control group. The same was true for the triacylglycerols TAGs, low density lipoprotein LDL, and the very low density lipoprotein VLDL and the KMP group was the same as the control. The high density lipoprotein HDL was significantly declined in all treatment groups except the KMP comparing with the control at (Pd"0.05). The serum hepatic enzymes; the alanine amino transferase ALT, the aspartate aminotransferase AST and the alkaline phosphatase ALP were all significantly elevated in all treatment groups accept the KMP group comparing with the control group. The serum creatine was significantly elevated only in the CHO group comparing with control group at (Pd"0.05); table 4.

Table 3. Comparison among kaempferol and statins effects on lipid profile of rats

Groups	TC(mg/dl)	TAGs (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
C	126 ± 3.3 ^{c}	68.4 ± 3.13 ^{d}	20.2 ± 1.1 ^{a}	90.4 ± 2 ^{c}	12.4 ± 1.2 ^{d}
CHO	161.8 ± 5.22 ^{a}	134.2 ± 2.7 ^{a}	10.2 ± 1.3 ^{b}	121.2 ± 2.1 ^{a}	26.2 ± 1.3 ^{a}
ATST	135 ± 5.11 ^{b}	124.6 ± 2.5 ^{b}	12.2 ± 1.4 ^{b}	100.2 ± 3.1 ^{b}	22.2 ± 1.7 ^{b}
SVST	136.6 ± 2.97 ^{b}	102.4 ± 2.2 ^{c}	12 ± 1.3 ^{b}	102 ± 3.2 ^{b}	20.2 ± 1.4 ^{c}
KMP	125 ± 1.11 ^{c}	64.2 ± 3.22 ^{e}	20 ± 3.2 ^{a}	91 ± 4.2 ^{c}	12 ± 2.1 ^{d}
LSD	10	4.2	7.8	9.2	2

Groups: C=Control, CHO=Cholesterol, ATST= Atorvastatin, SVST= Simvastatin, KMP= Kaempferol. LSD= Least Significant Difference. Similar small letters on number; a, b, c refer to presence of significance or not among groups.

Table 4. Comparison among kaempferol and statins effects on hepatic enzymes and creatine of rats

Groups	AST(U/L)	ALT(U/L)	ALP(U/L)	Creatine(g/dl)
C	21 ± 1.13 ^{d}	34.6 ± 3.13 ^{c}	237 ± 6.12 ^{b}	1.7 ± 0.4 ^{b}
CHO	68 ± 4.12 ^{b}	58.8 ± 5.31 ^{b}	299.2 ± 10.14 ^{a}	2.8 ± 0.5 ^{a}
ATST	86.8 ± 7.3 ^{a}	52.8 ± 4.23 ^{b}	278.4 ± 10.21 ^{a}	1.5 ± 0.2 ^{b}
SVST	54.4 ± 9.13 ^{c}	90.8 ± 7 ^{a}	279.4 ± 10.1 ^{a}	1.6 ± 0.6 ^{b}
KMP	30.4 ± 2.21 ^{d}	43.6 ± 6.24 ^{c}	248.2 ± 9.91 ^{b}	1.4 ± 0.3 ^{b}
LSD	18.8	9.2	30.2	1.1

Groups: C=Control, CHO=Cholesterol, ATST= Atorvastatin, SVST= Simvastatin, KMP= Kaempferol. LSD= Least Significant Difference. Similar small letters on number; a, b, c refer to presence of significance or not among groups.

DISCUSSION

Flavonols including the kaempferol were documented by researchers to be of high antioxidant features and have the ability to protect different body systems and to ameliorate bodily functions.[14,15] Our results of this study come as a confirmation to previous studies which have documented the sequelae of statins[16-18] and the advantages of flavonols.[19-21] The declination in the erythrocytes count, leukocytes count elevation, lipoproteins disturbances and hepatic enzymes disturbances which were noticed in our studies might be due the oxidative stress which is caused by the statins and hence the related reactive species of oxygen which were documented to be the major cause of cellular membranes damages and lipids peroxidation.[22-24] On other hand, the amelioration in the lipid profile, erythrocytes, hemoglobin, leukocytes, platelets and hepatic enzymes which were caused by kaempferol might be due to the great feature of kaempferol as antioxidant which render it to be affective protector to the cellular membranes, good modulator to the immunity, good modulator to the cellular pathways of healing and apoptosis and others.[25-27] Histological study involving the effects of the statins Atorvastatin and Simvastatin comparing with Kaempferol on organs of rats such as liver, kidney and spleen is recommended to support our findings.

CONCLUSIONS

It is obvious based upon our results in this study that Kaempferol is safer than Atorvastatin and Simvastatin when it is used to alleviate the hyperlipidemia; where it does not affect the blood indices such as hemoglobin, erythrocytes, leukocytes and others. Besides, kaempferol does not affect the hepatic enzymes, alkaline phosphatase and creatine. We can recommend the Kaempferol to be used as antihyperlipidemic medicine. Hyperlipidemia is a worldwide common disorder, so the use of Kaempferol would be a good choice considering its lacking the side effects which are caused by Atorvastatin and Simvastatin.

Conflict of interest

No conflict of interest.

REFERENCES

1. Tripathi DM, Vilaseca M, Lafoz E, et al (2018). Simvastatin prevents progression of acute on chronic liver failure in rats with cirrhosis and portal hypertension. Gastroenterology. 155(5): 1564-1577. Doi10.1053/j gastro.
2. Brunton LL, Dandan RH, Knollmann BC (2018). Goodman & Gilman's, The Pharmacological Basis of Therapeutics. Thirteenth edition, USA. Pp: 609.
3. Jabbar AA, Al Uboody WSH, Waad SK, Abbas MQ (2018). Physiological Study about the Hematological Effects of Rosuvastatin and Lovastatin as Compared with Sophoretin in Rats (Rattus Norvegicus). Journal of Global Pharma Technology, 10(08):314-318.
4. Hashim WS, Mshary GS, Al-Samawy ERM, Waad SK (2019). Physiological Study about Rosuvastatin and Lovastatin as Compared with Quercetin in Rats (Rattus norvegicus). Journal of Global Pharma Technology, 11(05) (Suppl.): 28-32.
5. Flamini R, De Rosso M (2008). Polyphenols analysis by liquid-mass spectrometry. In: Hyphenated Techniques in Grape and Wine Chemistry, John Wiley & Sons Ltd, Hoboken, NJ, USA, pp. 81–128.
6. Niering P, Michels G, Wätjen W, Ohler S, Steffan B, Chovolou Y, Kampkötter A, Proksch P, Kahl R (2005). Protective and detrimental effects of kaempferol in rat H4IIE cells: Implication of oxidative stress and apoptosis. Toxicology and Applied Pharmacology, 209 (2): 114-122. https://doi.org/10.1016/j.taap.2005.04.004.
7. Al-Numair KS, Chandramohan G, Veeramani C, Alsaif MA (2015). Ameliorative effect of kaempferol, a flavonoid, on oxidative stress in streptozotocin-induced diabetic rats. Redox Report, 20:5, 198-209, DOI: 10.1179/1351000214Y.0000000117.
8. Garde-Cedran T, Gonzalo-Diago A (2016). Kaempferol Biosynthesis, Food Sources and Therapeutic Uses. Hauppauge, New York: Nova Science Publisher's, Inc., Series: Biochemistry research trends.
9. Subcommittee of laboratory animal nutrition, committee on animal nutrition, board in agriculture, and National research council (1995) Nutrient requirements of laboratory Animals, Fourth revised editition, National academy press.
10. Karam I, Ma N, Yang Y-J, Li J-Y (2018). Induce Hyperlipidemia in Rats Using High Fat Diet Investigating Blood Lipid and Histopathology. J Hematol Blood Disord 4(1):104.

11. Atorvastatin Data Sheet (2022). https://www.msd.com/docs/product/safety-data-sheets/hh-sds/Atorvastatin%20Formulation_HH_ZA_6N.pdf.
12. Hong Y, Dai DP, Cai JP, et al. (2022). Effects of Simvastatin on the Metabolism of Vonoprazan in Rats Both in vitro and in vivo. Drug Design, Development and Therapy, 16: 1779–1789. https://doi.org/10.2147/DDDT.S365610.
13. Somayeh K, Adele J, Parvin B (2018). Kaempferol attenuates cognitive deficit via regulating oxidative stress andneuroinflammation in an ovariectomized rat model of sporadic dementia. Neural Regeneration Research, 13(10): 1827-1832. doi: 10.4103/1673-5374.238714.
14. Al-Uboody WS, Al-Diwan MA (2015). Ameliorating and protective role of quercetin against o-anisidine toxicity on some reproductive aspects of laboratory male rats (Rattus norvegicus). Bas. J. Vet. Res, 14(1): 250-261.
15. Al-Uboody WS, Al-Diwan MA (2015). Study the protective role of quercetin against o-anisidine toxicity on some hematological parameters of laboratory male rats (Rattus norvegicus). Bas. J. Vet. Res, 14(1): 262-273.
16. Vasudevan AR, Hamirani YS, Jones H P (2005). Cleveland clinical journal of medicine, 72: 11.
17. Gillett RC, Norrell A (2011). Considediets for safe use of statins: liver enzyme abnormalities and muscle toxicity. American Family Physician. 83: 6.
18. Taleb HM, Almasri MI, Siam IN, Najim AA, Ahmed IA (2014) The effect of rosuvastatin on liver function among patients with coronary heart disease in gaza strip. Pharmacology and Pharmacy, 5: 781-788.
19. Ricardo SFK, De Oliveira TT, Nagem JT (2001) Effect of flavonoids morin; quercetin and nicotinic acid on lipid metabolism of rats experimentally fed with triton. Brazilian archives of biology and technology, 44 (3): 263-267.
20. Duchnowicz P, Broncel M, Podsedek A, Koter-Michalak M (2012) Eur. J. Nutr., 51:435-443.
21. Jeong MS, Kang JM, Choi NH, Kim HJ, Kim IJ (2012) Quercetin ameliorates hyperglycemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice. Nutrition Research and Practice, 6(3):201-207.
22. Brownlee M (2001). Biochemistry and molecular cell biology of diabetic complications. Nature 414:813–20.
23. Hazarika A, Sarkar SN (2001). Effect of isoproturon pre-treatment on the biochemical toxicodynamics of anilofs in male rats. Toxicology 165:87–95.
24. Maritim AC, Sanders RA, Watkins JB (2003). Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 17:24–38.
25. Kim JM, Lee EK, Kim DH, et al. (2010). Kaempferol modulates proinflammatory NF-kappa B activation by suppressing advanced glycation end products-induced NADPH oxidase. Age (Dordr.) 32: 197–208.
26. Nguyen TT, Tran E, Ong CK, et al. (2003). Kaempferol-induced growth inhibition and apoptosis in A549 lung cancer cells is mediated by activation of MEK-MAPK. J Cell Physiol 197:110–21.
27. Lee YJ, Suh KS, Choi MC, et al. (2010). Kaempferol protects HIT-T15 pancreatic beta cells from 2-deoxy-D-ribose-induced oxidative damage. Phytother Res 24:419–23.