European Review for Medical and Pharmacological Sciences 2023; 27: 4221-4231

Beneficial effects of Naringin against lopinavir/ritonavir-induced hyperlipidemia and reproductive toxicity in male albino rats

V. KRISHNARAJU[1], Y. ALGHAZWANI[1], S. DURGARAMANI[2], Y.I. ASIRI[1], K. PRABAHAR[3,4], K. KALPANA[5], V. RAJALAKSHIMI[1], A.K. NOOHU[7], P. PREMALATHA[6], S.A. SIRAJUDEEN[7], V. KUMAR[8], V. VINOTH PRABHU[3]

[1]Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Asir, Saudi Arabia
[2]Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan, Saudi Arabia
[3]Department of Pharmacy Practice, Faculty of Pharmacy, University of Tabuk, Tabuk, Saudi Arabia
[4]Department of Pharmacy Practice, Faculty of Pharmacy, Dr. M.G.R. Educational and Research Institute, Velappanchavadi, Chennai, Tamil Nadu, India
[5]Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, India
[6]College of Nursing, Mahalah Branch for Girls King Khalid University, Asir, Saudi Arabia
[7]Department of Clinical Pharmacy, College of Pharmacy, King Khalid University, Abha, Saudi Arabia
[8]Department of Pharmaceutical Chemistry, College of Pharmacy, King Khalid University, Asir-Abha, Saudi Arabia

Abstract. – OBJECTIVE: This research work was planned to determine whether Naringin (NG) had any protective effects against lopinavir/ritonavir (LR)-induced alterations in blood lipid levels, hepatotoxicity, and testicular toxicity.

MATERIALS AND METHODS: Four groups of six rats each were used for the study: Control (1% ethanol), naringin (80 mg/kg), lopinavir (80 mg/kg)/ritonavir (20 mg/kg), and lopinavir (80 mg/kg)/ritonavir (20 mg/kg) + naringin (80 mg/kg). The drug treatment was continued for 30 days. On the last day, the serum lipid fractions, liver biochemical parameters, testicular antioxidants (enzymatic and non-enzymatic), and the histopathology of the liver and testis tissue were assessed for all rats.

RESULTS: Treatment with NG decreased significantly ($p<0.05$), the baseline serum levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and increased high-density lipoprotein cholesterol (HDL-C). But these parameters were significantly ($p<0.05$) increased in LR-treated animals. Naringin, co-administered with LR, restored the liver and testicular biochemical, morphological, and histological balance.

CONCLUSIONS: This study shows that NG can be used as a treatment for LR-induced biochemical and histological changes in the liver and testes and changes in serum lipid levels.

Key Words:
Naringin, Lopinavir/ritonavir, Drug-induced hyperlipidemia, Protease inhibitors, Anti-retroviral regimens.

Introduction

The standard regimens needed for the treatment of human immunodeficiency virus (HIV) infection include protease inhibitors (PIs), which are crucial. However, harmful effects such as dyslipidemia, hepatotoxicity, and testicular oxidative damage have been associated[1-3] with the clinical usage of PIs containing anti-retroviral regimens. Changes in plasma lipid levels are common class-related side effects of protease inhibitors, which may restrict their usage, particularly in patients with a history of cardiovascular risk. Lopinavir/ritonavir (LR) treatment may be associated with more significant and frequent abnormalities in plasma lipids compared to other proteases inhibitors[4,5]. According to studies[6], the two main characteristics of modifications in plasma lipids with LR therapy are decreased high-density lipoprotein cholesterol (HDL-C) and raised plasma triglycerides (TG). Additionally, with

Corresponding Author: Krishnaraju Venkatesan, MD; e-mail: kvenkatesan@kku.edu.sa

the usage of LR, elevations in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were frequently noted. Oxidative stress and malondialdehyde serum levels may influence lipid levels[7].

Additionally, LR-containing treatments were found to have an average occurrence of 10% hepatotoxicity across PI-containing regimes[1]. A common sign of liver damage caused by LR is abnormal hepatic serum clinical chemistry, which worsens over time[8]. Additionally, abnormalities in hepatic morphology, including ballooning of hepatocytes, severe necrosis, and distortion in hepatocyte and sinusoidal radial patterns, were seen in animals[9]. Additionally, hepatic abnormalities in humans have been documented[10]. These include sinusoidal degeneration, hepatocyte necrosis, steatosis, mitochondrial dysfunction, and blockage of sinusoidal lumina. According to research done on animals, oxidative stress (OS), lipid peroxidation (LPO), and nitrosative stress (NS) may also play a role in the described hepatic change caused by LR. Adaramoye et al[3] also observed that LR might harm the health of the male reproductive system, particularly in the structural integrity of the testes and the quality of sperm.

Citrus fruits, cherries, tomatoes, cocoa, and grapefruit contain flavone naringin (NG). This substance is a nontoxic natural product with numerous benefits, including anti-cancer, anti-inflammatory, antioxidant, nephron and hepatoprotective, lipid-lowering, testicular, and cardiovascular protective qualities[11,12]. In light of the above benefits of NG, the current investigation aims to evaluate the protective effect of NG against dyslipidemia and testicular dysfunction brought on by LR in male Wistar rats. It is noteworthy that the research was carried out using histology and biochemical techniques.

Materials and Methods

Experimental Animals and Treatment

A total of 24 male albino rats weighing 210±15 g were obtained from the Central Animal House Facility, King Khalid University, Kingdom of Saudi Arabia. The King Khalid University's Institutional Ethics Committee accepted the research, and the animals were kept in regular laboratory settings with access to standard laboratory feed, tap water, and a 12/12-hour light cycle (Approval No.: ECM/2022/2903). Four groups of six animals each were created randomly: Control, Naringin, Lopinavir/Ritonavir, and Lopinavir/Ritonavir + Naringin (LRNG). One percent ethanol was given to the control group. The 80 mg/kg lopinavir and 20 mg/kg ritonavir dosage for the lopinavir/ritonavir (Alltera, Mylan Pharmaceuticals Pvt Ltd, Canonsburg, PA, USA) group. An 80 mg/kg dosage of Naringin (Sisco Research Laboratories Pvt. Ltd., Chennai, India) was given to NG group. Identical amounts of each drug were administered to the combination group. Rats were given LR and NG using the dosage and route described in the earlier reports[13,14]. In 1% ethanol, the test material was dissolved. To minimise changes in metabolism linked to the circadian rhythm, each drug was given daily as one percent ethanol by gavage for 30 days between 8 and 10 in the morning.

Collection of Samples

Using diethyl ether, rats were sacrificed after receiving treatment for 30 days. Through heart puncture, blood samples were obtained and put into sterile sample containers. The blood samples were centrifuged at 1,200 rpm for 15 minutes after being allowed to clot. The serum was collected, and the lipid profile was assessed. According to Afolabi et al[15] evaluation of serum TC, TG, LDL-C, VLDL-C, and HDL-C levels was conducted. The liver and testes of rats were removed, weighed, and examined for histological abnormalities.

Preparation of Tissue Homogenate

The liver tissues were homogenized using a buffer containing 0.1 percent Triton X-100 (pH 7.4). The homogenate was centrifuged for 30 minutes at 12,000 rpm and 4°C, and the supernatant served as the sample for biochemical research[16]. To produce the post-mitochondrial fraction (PMF) of the testes, which was employed for biochemical analysis, the testes tissue samples were homogenized in 50 mM phosphate buffer (4 volumes) at pH 7.4 and centrifuged for 15 min at 10,000 g^3.

Evaluation of Liver Biochemical Parameters

Aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and gamma-glutamyl transferase (GGT) were measured in the homogenate using standard test kits following manufacturer's instructions after centrifugation[17].

Evaluation of Liver Malondialdehyde and Superoxide Dismutase

According to Draper and Hadley[18] and Beauchamp and Fridovic[19], the liver's superoxide dismutase (SOD) and malondialdehyde (MDA) levels, respectively, were determined.

Measurement of Biochemical Parameters in the Rat Testicles

The testicular glutathione (GSH), catalase, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione S-transferase (GST) levels were measured using the method of Ahangarpour et al[20], Alboghobeish et al[21] , Ahangarpour et al[22], Lawrence RA[23], and Habig et al[24] respectively.

Histology of the Liver and Testes

After being sliced into a slab of 0.5 cm thickness and preserved in Bouin's solution for 24 hours, the liver was dehydrated using 70% alcohol. Liver tissues were embedded in moistened paraffin wax after being put through 90% alcohol and chloroform at various periods. Five-micron serial sections were cut. Wax was removed from prepared liver tissue slides using pure alcohol. Then, hematoxylin and eosin were used to stain the slides. The slides underwent light microscopy inspections, and pertinent sections were snapped[25].

Statistical Analysis

The GraphPad Prism 5 (La Jolla, CA, USA) statistical tool was used for the statistical analysis, and the ANOVA test was used to compare the means of the different groups. Results are given as the mean±the standard error of the mean (SEM). The cut-off for statistical significance was $p<0.05$.

Results

Treatment with NG significantly ($p<0.05$) decreased baseline serum levels of TG, TC, LDL-C, and VDL-C and increased HDL-C compared to the control. Pronounced and significant ($p<0.05$) decreases in TC, TG, LDL-C, and VLDL-C and significant ($p<0.05$) increases in HDL-C to 55.46±3.12, 39.27±3.46, 13.2±0.48, 15.38±0.06 and 29.4±2.6 mg/dl respectively were obtained in rats treated with a combination of LR and NG when compared to LR (Table I). On the contrary, treatment with LR significantly ($p<0.05$) increased serum levels of TG, TC, LDL-C, VDLC, and decreased HDL-C when compared to the control (Table I).

AST, ALP, ALT, GGT, and LDH are all in the liver. With the introduction of hepatic morphological changes brought on by xenobiotics, the hepatic content of the parameters mentioned above can be changed. When compared to the control, LR treatment in the present study led to substantial ($p<0.05$) increases in the liver's AST, GGT, ALT, ALP, and LDH levels (Table II). The observation confirms that LR therapy results in liver damage. However, compared to rats treated with LR, the parameters mentioned above were considerably reduced in rats treated with NG ($p<0.05$) both alone and in combination with LR ($p<0.05$) (Table II).

Compared to the control group, animals exposed to LR also displayed a significant ($p<0.05$) rise in MDA and decreased SOD levels. Comparing the treatment with NG to the control, there is no difference in the MDA and SOD levels. In comparison to the LR, there was a substantial ($p<0.05$) decrease (28.4±2.18) in MDA and a rise (25.2±2.42) in SOD level when the combination (NG+LR) was administered (Table III).

Table I. Effects of treatments with lopinavir/ritonavir, Naringin, and co-administered lopinavir/ritonavir + Naringin on baseline serum lipid levels of male albino rats.

	Lipid Profile (mg/dl)				
	TG	TC	LDL-C	VLDL-C	HDL-C
Control	44.22±2.24	65.49±3.56	17.09 ± 1.54	12.28 ± 0.32	27.3±2.4
Naringin	34.89±1.24*	46.09±2.81*	11.12 ± 0.04*	9.32 ± 0.45*	28.5±2.6*
Lopinavir/ritonavir	119.3±5.58*	135.3±3.66*	57.49 ± 3.14*	44.74 ± 3.16*	25.4±1.7*
Lopinavir/ritonavir + Naringin	39.27±3.46**	55.46±3.12**	13.2 ± 0.48**	15.38 ± 0.06**	29.4±2.6**

Results are expressed as mean ± SEM. *Significant ($p<0.05$) difference when compared to control. **Significant ($p<0.05$) difference when compared to LPV/r.

Table II. Effects of treatments with lopinavir/ritonavir, Naringin, and co-administered lopinavir/ritonavir + Naringin on biochemical parameters in the liver of male albino rats.

	Biochemical parameters in the liver				
	AST	ALT	ALP	GGT	LDH
Control	249.7±14.9	226.6±12.4	246.2±18.6	27.6±2.84	237.5±14.7
Naringin	243.5±12.7	218.2±14.5	232.4±18.4	24.2±3.83	232.5±12.4
Lopinavir/ritonavir	548.2±14.4*	478.9±16.8*	612.4±36.2*	474.0±32.22*	488.2±26.4*
Lopinavir/ritonavir + Naringin	254.4±14.2**	247.6±16.5**	274.2±22.4**	42.3±4.23**	261.6±14.6**

AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transferase; LDH, Lactate dehydrogenase; Values are expressed as M±SEM. *Significant (p<0.05) difference when compared to the control. **Significant (p<0.05) difference when compared to lopinavir/ritonavir.

Table III. Effect of lopinavir/ritonavir, Naringin, and co-administered lopinavir/ritonavir + Naringin on MDA (nmole/g tissue) and SOD (units/mg protein) in rats.

	Biochemical parameters in the liver	
	MDA	SOD
Control	22.47±1.42	29.2±1.32
Naringin	24.27±0.18	27.3±2.24
Lopinavir/ritonavir	39.7±2.86*	19.4±2.22*
Lopinavir/ritonavir + Naringin	28.4±2.18**	25.2±2.42**

Results are expressed as mean±SEM. *Significant (p<0.05) difference when compared to the control. **Significant (p<0.05) difference when compared to lopinavir/ritonavir.

The GSH-Px enzyme was reduced after LR treatment (p<0.05), while this factor increased in those who had received NG-LR treatment. Following the LR treatment, the GSH level decreased; however, after NG-LR ingestion, it increased (p<0.05). SOD and CAT levels, on the other hand, significantly increased following treatment with NG and decreased after treatment with LR, respectively (p<0.05) (Table IV). The GST level considerably (p<0.05) increased in the NG-LR co-treatment group (65.18±1.94) and reduced in the LR group (44.21±1.91). However, the levels in the NG group (70.24±1.84) were the same as the control group (69.12±2.02).

Table IV. Effect of lopinavir/ritonavir, Naringin, and co-administered lopinavir/ritonavir + Naringin on enzymatic and non-enzymatic antioxidants in rat testicles.

	Biochemical parameters in the liver				
	GSH-Px (µmol/mg of protein)	Catalase (µmol/mg of protein)	GSH (µg/mg of protein)	SOD (U/mg of protein)	GST (nmol/min/mg of protein
Control	2.5±0.38	63.16±1.27	0.74±0.11	2.33±0.49	69.12±2.02
Naringin	2.6±0.76	64.18±1.64	0.86±0.06	2.57±0.21	70.24±1.84
Lopinavir/ritonavir	1.08±0.42*	44.2±3.4*	0.72±0.06*	1.42±0.52*	44.21±1.91
Lopinavir/ritonavir + Naringin	2.12±0.06**	56.2±2.5**	0.85±0.05**	2.29±0.46**	65.18±1.94**

Results are expressed as mean±SEM. *Significant (p<0.05) difference when compared to control. **Significant (p<0.05) difference when compared to lopinavir/ritonavir.

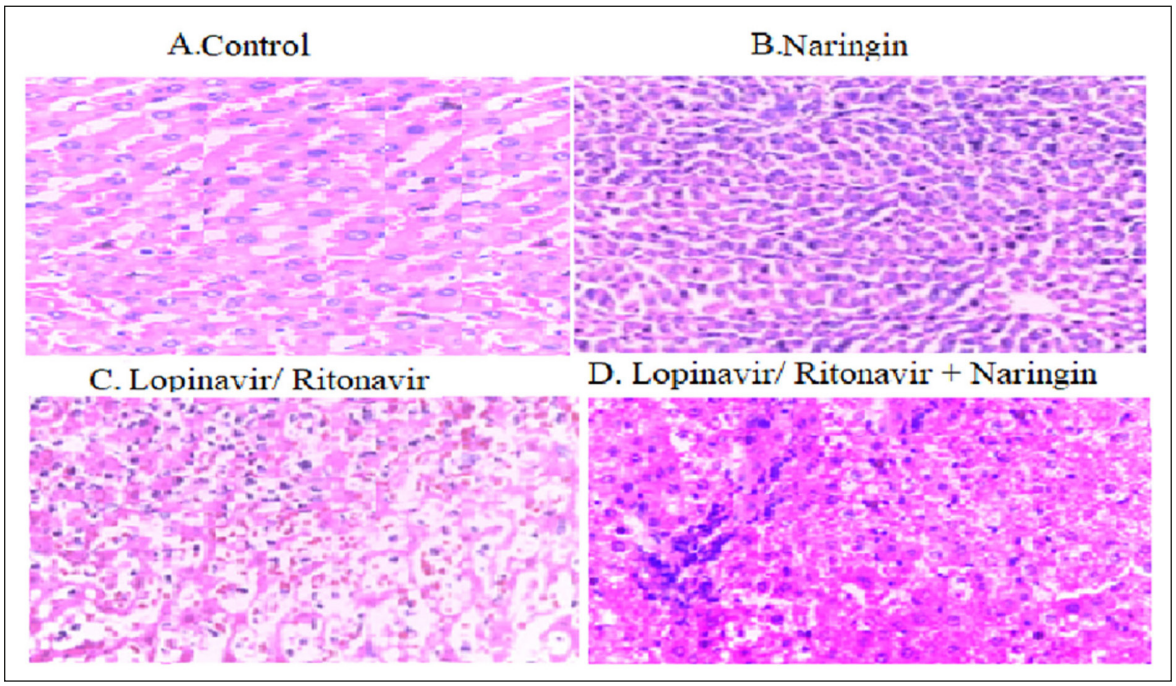

Figure 1. The above figures (**A-D**) are micrographs of the H and E stained sections of the liver of the control rats and the liver of rats in the experimental groups. Normal hepatocytes were observed in the liver of control rats (**A**). In addition, normal hepatocytes were observed in the liver of rats treated with Naringin and a combination of lopinavir/ritonavir + Naringin (**B-D**). On the other hand, extensive hepatocyte necrosis was observed in the liver of lopinavir+ritonavir-treated rats (Mag. x400).

The livers of the control rats in the current study's histological analysis displayed normal histology (Figure 1A). Additionally, NG animals revealed normal liver histology (Figure 1B). However, substantial hepatocyte necrosis was seen in the liver of rats treated with LR (Figure 1C). However, rats co-administered with LR+NG had no hepatocyte necroses in their livers (Figure 1D).

There were no differences between the control (A: Group I) and NG (B: Group II) groups when H and E stained testis sections were compared. They demonstrated that the testis comprises seminiferous tubules surrounded by a basement membrane (BM) and that interstitial tissue is filled with many Leydig cells between the tubules. The inside of seminiferous tubule is lined by Sertoli and Germ Cells. The lumen of the seminiferous tubules contains spermatozoa (Figures 2A-B). Group III (LR treated) sections showed seminiferous tubules divided into broad gaps with uniform acidophilic material and few interstitial cells. The tubules were partially or entirely detached from the BM (Figure 2C).

Detachment of the seminiferous epithelium resulted in the observation of completely distorted, irregularly shaped tubules. In other tubules, the seminiferous epithelium was separated by vast gaps. Along with diminished interstitial cells replaced by homogenous acidophilic substances, dilated congested blood vessels were also visible (Figure 2C). The most notable observation in Group III was complete cytoplasm loss, mainly in basal cells with tiny, highly black pyknotic nuclei, whereas other cells displayed transparent cytoplasm around the nucleus.

Many vacuoles were found on the BM, which separates the BM from the rest of the seminiferous epithelium. Small acidophilic entities were mostly discovered in the tubules' basal region. Most tubules' lumens are empty or only contain a small amount of sperm. Most of these sperm were tightly packed between the tubular basal cells. A majority of the tubules had two layers of cells lining them, and the seminiferous epithelium was destroyed (Figure 2C). Interstitial cells also showed up with vacuolated cytoplasm. The histological image improved in Group IV (LR+NG) specimens, as evidenced by smaller gaps between seminiferous tubules that nevertheless, include intact interstitial tissue and Leydig cells. With spermatogonia at various development stages, the seminiferous epithelium virtually recovered its usual height (Figure 2D).

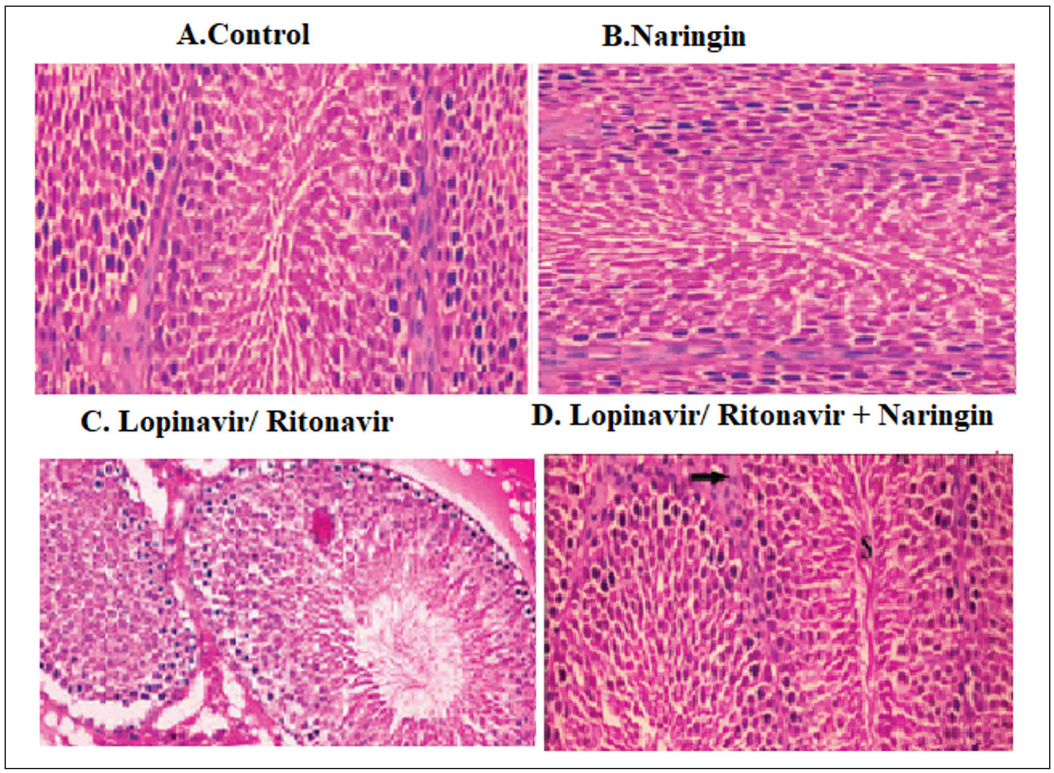

Figure 2. Effect of lopinavir/ritonavir, Naringin and co-administered Lopinavir/ritonavir + Naringin on testicular histopathology (H&E x400). Figures (**A-D**) are micrographs of the H and E stained sections of the testicles of the control rats and the experimental groups.

Discussion

Ritonavir's primary function in the combination medication of LR is to enhance lopinavir *via* inhibiting CYP3A4. SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) and the related coronavirus illness (COVID-19) have been successfully treated with this combination. On ClinicalTrials.gov, as of July 16, 2020, 59 studies involving people with LR in COVID-19 have accrual targets of more than 16,000 patients[26]. Furthermore, the P1060 trials demonstrated that, in terms of risk of treatment failure, LR-based regimens were superior to nevirapine (NVP)-based regimens for HIV-infected children under three years old. Therefore, both World Health Organization (WHO) and United States (US) treatment guidelines[27] currently propose an LR-based regimen for HIV-infected children under the age of three. However, recognized risk factors for atherosclerotic cardiovascular disease, such as hypercholesterolemia, metabolic syndrome, and hypertriglyceridemia[27,28], as well as testicular toxicity[3,29], have all been linked to LR. Hyperlipidemia caused by protease inhibitors is a growing concern as more patients use protease inhibitors (PI) regimens. So, in lopinavir/ritonavir (LR) treated rats, we set out to investigate the potential protective effects of Naringin against hyperlipidemia, hepatotoxicity, and testicular toxicity.

An independent risk factor for the emergence of atherosclerosis disease is lipoprotein. LDL is primarily formed when VLDL is broken down. The maximum amount of cholesterol and phospholipids (PL) can be carried by LDL. Additionally, it has been proposed that atherogenesis is caused by oxidatively changed LDL instead of unmodified LDL. Numerous cytokines, immune cell chemoattractant proteins, and growth factors are produced more readily when LDL is oxidized. Additionally, they cause platelet aggregation to rise, aggravating the lesion and thickening the artery wall. While HDL exhibits a negative correlation, elevated LDL and CAD show a positive correlation. In addition to facilitating the movement of cholesterol from peripheral tissues to the liver, where it is metabolized and eliminated from the body, HDL also inhibits the uptake of LDL from the artery wall[30].

The baseline serum concentrations of TG, TC, LDL-C, and VLDL-C were reduced in the current study by NG therapy, while HDL-C was elevated. This finding is in line with Jutric et al[31] who administered NG to mice for ten days and noted drops in serum lipid levels[31]. The outcome is also in line with the findings of Jeon et al[32] who claimed that Naringin has a potent lipid-lowering effect in hyperlipidemic rabbits. On the other hand, therapy with LR increased TG, TC, LDL-C, and VLDL-C and lowered HDL-C levels in the serum. This finding is consistent with research by Pistell et al[33] who administered LR to mice and noted increases in lipid and glucose levels. Additionally, Canavaghi et al[34] found increases in serum lipid and glucose levels after giving 30 mg/kg ritonavir, which is consistent with the findings of the current investigation.

In the current study, sterol regulatory element binding protein (SREBP) 1 and 2 activation-induced increases in cholesterol and fatty acid synthesis in the adipose tissue and liver were likely responsible for lipid parameters. Another potential mechanism is the suppression of triglyceride-rich lipoprotein remnant clearance by LR which inhibits two enzymes involved in lipid metabolism, namely lipoprotein lipase and lecithin cholesterol acyltransferase (LCAT). Additionally, LR can increase the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, increasing the production of lipoproteins[35]. The suppression of the 20S proteasome, which results in a reduction in the degradation of nascent apolipoprotein B, may be related to the LR-induced rise in lipid levels, according to studies[36] in cultured liver cells.

In addition, decreased HMG-CoA reductase activity[37] and downregulation of the triglyceride cell transporters, which is a fatty acid binding protein (FABP1) and CD36 in hepatocytes[38] may be responsible for the amelioration of LR-induced elevations in lipid levels in rats co-treated with NG.

The enzyme that controls the rate of cholesterol biosynthesis is HMG-CoA reductase. An increase in HMG-CoA reductase activity causes excessive cholesterol production and build-up, which causes foam cells to form, which is a necessary precursor to the development of atherosclerosis[30]. One explanation for the lower lipid level in NG-treated animals could be that HMG-CoA reductase inhibitors are known to reduce the secretion of VLDL and LDL levels. Additionally, it has been demonstrated[30] that NG increases the activity of the lipoprotein lipase (LPL) and lecithin cholesterol acyl transferase (LCAT) in rat plasma. LCAT is an HDL-associated enzyme and an essential component of extracellular cholesterol metabolism. LPL hydrolyses circulating TG-rich lipoproteins such as VLDL and chylomicrons. The hypocholesterolemic effect of NG may have resulted from elevated LCAT and LPL activity. The Farnesoid X receptor (FXR), a ligand-activated transcription factor essential for the expression of numerous proteins and biosynthetic enzymes necessary for the physiological maintenance of cholesterol, was also demonstrated[31,39] to be upregulated by Naringin. Additionally, it was shown that naringenin, a metabolic by-product of Naringin, modulates the 20S proteasome activity, which was previously found[40] to be increased in LR-treated rats.

According to several published observations[41-44], the elevated levels of ALT, AST, ALP, GGT, and LDH found in our investigation for the animals given LR are consistent. The observation confirms that LR therapy results in liver damage[45,46]. However, compared to rats treated with LR, the parameters mentioned above were significantly reduced in rats treated with NG alone ($p<0.05$) and in combination with LR ($p<0.05$) (Table II). Because these enzymes are located in the cytoplasmic region of the cell and are liberated into circulation in case of cellular injury, the increase in serum levels of hepatic markers seen in this study may be related to liver injury[47]. The NG protective effect found in this study is comparable to publications[37] that revealed considerably higher levels of SOD, CAT, and GSH in the liver of LDL receptor knockout mice. However, Naringin has been shown[31] to modify and restore key hepatic indicators of lipid metabolism and oxidative stress [Acyl-coenzyme A oxidase 1 (ACOX1), peroxisome proliferators-activated receptor alpha PPAR alpha), and peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 alpha)] to levels close to control.

Increased MDA and a decrease in SOD in the lopinavir/ritonavir-treated rats are significant indicators of induced oxidative stress and lipid peroxidation, which can be linked to the production of free radicals by this drugs[47]. The findings corroborate sure researchers' reported observations[29,48]. Compared to the LR group, Naringin administered to LR resulted in a considerable reduction in MDA and an increase in SOD (Table III). Rat study findings confirm that Naringin inhibits pro-inflammatory cytokines by boosting SOD activity, reducing apoptosis, inflammation, oxidative DNA damage, and autophagy[31,49,50]. According to earlier research, HIV PI-induced en-

doplasmic reticulum (ER) stress response and ensuing activation of the unfolded protein response serve as crucial cellular signaling mechanisms for the development of hepatotoxicity and metabolic syndromes[47]. As seen in this study, ER stress has been linked to the generation of reactive oxygen species, which can increase MDA levels and reduce SOD levels. The effect of NG on MDA and SOD that has been seen, as well as the normalization of ACOX1 expression that has been reported[47] for NG, are likely crucial mechanisms for lowering oxidative stress and lipid damage. Additionally, NG reverses hepatic structural alterations brought on by LR (Figure 1). The study of Adil et al[51] found that NG-restored liver structure in rats with acetaminophen intoxication can be associated with the protective effect of NG seen in our study. Additionally, the results of this investigation are consistent with the preventive effect of NG against CCL4-induced liver morphological changes in rats[52]. It is unclear how LR affects hepatic morphology. However, it could be due to OS, LPO, or the induction of an inflammatory cascade. Hepatic OS has been linked to LPO in the past in LR-treated rats, according to research[41]. OS is connected to the formation of oxidative radicals, which can cause various histopathological diseases, from hepatotoxicity to hepatocellular cancer, in a planned manner. It has been established[41] that enhanced free radical activity and concentration are frequently seen during hepatic cell injury caused by OS and LPO. Furthermore, the imbalance in the formation of free radicals and the existence of antioxidants contribute to oxidative stress. Molecular antioxidants, metallic chemical agents, and antioxidant enzymes are all components of the antioxidant defense system. SOD, CAT, and GPx are antioxidant enzymes that shield biological systems from the harmful effects of reactive oxygen species (ROS) and lessen their oxidative stress on testicular cell membranes[11]. When SOD activity is decreased, superoxide radicals build up and eventually block the CAT enzyme, and ROS levels rise when CAT activity is reduced.

Due to the production of ROS, LR enhanced oxidative stress in the rat testis, increasing lipid peroxidation and concurrently decreasing the activities of enzymatic (SOD, CAT, GPx, and GST) and non-enzymatic (GSH) antioxidant measures (Table IV). The antioxidant biomarkers GSH, GPx, GST, CAT, and SOD were considerably reduced in the testes of LR-treated rats in the current investigation, in line with earlier results[3]. However, the group that received NG treatment displayed elevated levels of antioxidant biomarkers. By conjugating with chemicals, the naturally occurring antioxidant GSH promotes detoxification, reduces free radical damage, and serves as a crucial cofactor for antioxidant enzymes such as GR, GPx, and GST[3]. The study's findings about the reduction of intracellular GSH indicated the toxic effects of LR, which could harm the testes. Antioxidant and glutathione levels are typically abnormally low in HIV-positive people[3]. Therefore, the relevance of NG-like therapy is necessary because the combined effects of LR therapy and HIV infection may further deplete the patients' GSH or antioxidant levels.

Overproduction of ROS causes oxidative stress in tissues, especially in cellular parts of phospholipids with polyunsaturated fatty acid residues that are vulnerable to oxidation[53]. Through a cyclization reaction, the peroxyl radicals created by the assault can be rearranged to create endoperoxides, which are intermediates of malondialdehyde (MDA)[3]. An increase in MDA levels was seen in LR-treated rats in a prior study[3], which was attributed to the testicular cells' diminished antioxidant capacity. Lipid peroxidation can cause various cell damage to essential macromolecules like DNA, membrane functions, and sperm quality impairment[3]. The presence of unsaturated fatty acids in the sperms' membranes makes them more vulnerable to lipid peroxidation[54]. According to the previously mentioned mechanism, one study's treatment[55,56] of LR in rats significantly reduced their epididymal sperm motility and counted. LR seriously disrupted the cytoarchitecture of the testes (Figure 2), which decreased the number of spermatogenic cells in the rats' seminiferous tubules. Our biochemical findings confirmed this histology finding.

On the other hand, NG has shielded the testes from toxicity brought on by LR. The protective effect of NG may be accomplished by boosting antioxidant biomarkers, preventing intracellular ROS formation by its impact on lipid peroxidation, and removing the ROS generated. The protective action of NG against ROS-induced cytotoxicity was demonstrated in earlier investigations employing carbon tetrachloride, arsenic, lead, cisplatin, or cadmium[11]. According to specific theories[57], the 5-hydroxy and 4-carbon groups in the NG's C ring may pair with Cu and Fe ions, lowering the ROS. Additionally, it has been noted[58] that the NG increases the expression of numerous genes associated with antioxidants and decreases the activity of

ROS-forming enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Additionally, in rats exposed to bisphenol A (BPA), NG was demonstrated[11] to increase testicular weight and volume, decrease testicular total protein level, increase sperm count, elevate LDH and ALP, increase plasma levels of Luteinizing hormone (LH), Follicle stimulating hormone (FSH), and testosterone, and normalize estradiol levels. This protective effect results from NG's direct or indirect impact on the testicular cells' oxidative damage.

Conclusions

Naringin co-treatment ameliorated LR-induced increases in serum lipid, alterations in hepatic morphology, and testicular toxicity. Based on the observations of this study, NG could be used to manage LR-associated alterations in serum lipid, liver, and testicular toxicity.

Data Availability

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Informed Consent

Not applicable.

Ethics Approval

The Institutional Animal Ethics Committee (IAEC) of King Khalid University in Abha, Saudi Arabia (ECM/2022/2903) approved all experimental procedures.

Funding

King Khalid University funded this study through the Large Research Group Project under grant number "RGP 2/89/43".

Authors' Contributions

Krishnaraju Venkatesan, Yahia Alghazwani, Durgaramani Sivadasan and Yahya I. Asiri conceived and designed research, conducted experiments, and Kousalya Prabahar, Kalpana Krishnaraju, Rajalakshimi Vasudevan wrote the manuscript, Noohu Abdulla Khan Premalatha Paulsamy, Sirajudeen Sheikh Alavudeen, Vinoth Prabhu Veeraman and Kumar Venkatesan done supervision of the work. All authors read and approved the manuscript.

Acknowledgements

The authors extend their sincere appreciation to the Deanship of Scientific Research at King Khalid University for funding this study through the Large Research Group Project under grant number "RGP 2/89/43".

ORCID ID

V. Krishnaraju: 0000-0003-2853-5907
P. Kousalya: 0000-0001-8224-4276
V. Rajalakshimi: 0000-0002-1173-7724

References

1) Sulkowski MS. Drug-induced liver injury associated with anti-retroviral therapy that includes HIV-1 protease inhibitors. Clin Infect Dis 2004; 38: 90-97.
2) Alvi RM, Neilan AM, Tariq N, Awadalla M, Afshar M, Banerji D, Rokicki A, Mulligan C, Triant VA, Zanni MV, Neilan TG. Protease Inhibitors and Cardiovascular Outcomes in Patients With HIV and Heart Failure. J Am Coll Cardiol 2018; 72: 518-530.
3) Adaramoye OA, Akanni OO, Adewumi OM, Owumi SE. Lopinavir/Ritonavir, an Antiretroviral Drug, Lowers Sperm Quality and Induces Testicular Oxidative Damage in Rats. Tokai J Exp Clin Med 2015; 40: 51-57.
4) Eron J, Yeni P, Gathe J, Estrada V, DeJesus E, Staszewski S, Lackey P, Katlama C, Young B, Yau L, SutherlandPhillips D, Wannamaker P, Vavro C, Patel L, Yeo J, Shaefer M. The Klean study: fosamprenavirritonavir versus lopinavir-ritonavir, each in combination with abacavir-lamivudine, for the initial treatment of HIV infection over 48 weeks: a randomised non-inferiority trial. Lancet 2006; 368: 476-482.
5) Hill A, Sawyer W, Gazzard B. Effects of first-line use of nucleoside analogues, efavirenz, and ritonavir-boosted protease inhibitors on lipid levels. HIV Clin Trials 2009; 10: 1-12.
6) Mulligan K, Grunfeld C, Tai VW, Algren H, Pang M, Chernoff DN, Lo JC, Schambelan M. Hyperlipidemia and insulin resistance are induced by protease inhibitors independent of changes in body composition in patients with HIV infection. J Acquir Immune Defic Syndr 2000; 23: 35-43.
7) Minhajuddin, M. Hypolipidemic and antioxidant properties of tocotrienol rich fraction isolated from rice bran oil in experimentally induced hyperlipidemic rats. Food Chem Toxicol 2005; 43: 747-753.
8) McGrowder DA, Miller F, Anderson Cross M, Anderson-Jackson L, Bryan S, Dilworth L. Abnormal Liver Biochemistry Tests and Acute Liver Injury in COVID-19 Patients: Current Evidence and Potential Pathogenesis. Diseases 2021; 9: 1-24.
9) Azu OO, Jegede AI, Ugochukwu O, Onanuga IO, Kharwa S, Naidu EC. Hepatic histomorphological and biochemical changes following highly active antiretroviral therapy in an experimental animal

model: Does Hypoxis hemerocallidea exacerbate hepatic injury? Toxicol Rep 2016; 3: 114-122.

10) Chwiki S, Campos MM, McLaughlin ME, Kleiner DE, Kovacs JA, Morse CG, Abu-Asab MS. Adverse effects of antiretroviral therapy on liver hepatocytes and endothelium in HIV patients: An ultrastructural perspective. Ultrastruct Pathol 2017; 41: 186-195.
11) Alboghobeish S, Mahdavinia M, Zeidooni L, Samimi A, Oroojan AA, Alizadeh S, Dehghani MA, Ahangarpour A, Khorsandi L. Efficiency of Naringin against reproductive toxicity and testicular damages induced by bisphenol A in rats. Iran J Basic Med Sci 2019; 22: 315-523.
12) Malakul W, Pengnet S, Kumchoom C, Tunsophon S. Naringin ameliorates endothelial dysfunction in fructose-fed rats. Exp Ther Med 2018; 15: 3140-3146.
13) Elsawy H, Alzahrani AM, Alfwuaires M, Abdel-Moneim AM, Khalil M. Nephroprotective effect of naringin in methotrexate induced renal toxicity in male rats. Biomed Pharmacother 2021; 143: 112180.
14) Khaled SS, Soliman HA, Abdel-Gabbar M, Ahmed NA, Attia KAHA, Mahran HA, El-Nahass ES, Ahmed OM. The Preventive Effects of Naringin and Naringenin against Paclitaxel-Induced Nephrotoxicity and Cardiotoxicity in Male Wistar Rats. Evid Based Complement Alternat Med 2022; 2022: 8739815.
15) Afolabi OK, Oyewo EB, Adekunle AS, Adedosu OT, Adedeji AL. Impaired lipid levels and inflammatory response in rats exposed to cadmium. EXCLI J 2012; 11: 677-687.
16) Nathiya S, Nandhini A. Evaluation of antioxidant effect of Salacia oblonga against aluminum chloride induced visceral toxicity in albino rats. Int J Basic Clin Pharmacol 2014; 3: 315-319.
17) Reitman S, Frankel S. A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. Am J Clin Pathol 1957; 28: 56-63.
18) Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990; 186: 421-431.
19) Beauchamp C, Fridovic I. Superoxide dismutase: improved assays and an assay applicable to acryl amide gels. Anal Biochem 1971; 44: 276-287.
20) Ahangarpour A, Alboghobeish S, Oroojan AA, Zeidooni L, Samimi A, Afshari G. Effects of Combined Exposure to Chronic High-Fat Diet and Arsenic on Thyroid Function and Lipid Profile in Male Mouse. Biol Trace Elem Res 2018; 182: 37-48.
21) Alboghobeish S, Mahdavinia M, Zeidooni L, Samimi A, Oroojan AA, Alizadeh S, Dehghani MA, Ahangarpour A, Khorsandi L. Efficiency of naringin against reproductive toxicity and testicular damages induced by bisphenol A in rats. Iran J Basic Med Sci 2019;22:315-523
22) Ahangarpour A, Oroojan AA, Rezae M, Khodayar MJ, Alboghobeish S, Zeinvand M. Effects of butyric acid and arsenic on isolated pancreatic islets and liver mitochondria of male mouse. Gastroenterol Hepatol Bed Bench 2017; 10: 44-53.
23) Lawrence RA. Reprint of “glutathione peroxidase activity in selenium-deficient rat liver” Biochem Biophysic Res Com 2012; 425: 503-509.
24) Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-7139.
25) Obianime AW, Roberts II. Antioxidants, cadmium-induced toxicity, serum biochemical and the histological abnormalities of the kidney and testes of the male Wistar rats. Niger J Physiol Sci 2009; 24: 177-185.
26) Lepage MA, Rozza N, Kremer R, Grunbaum A. Safety and effectiveness concerns of lopinavir/ritonavir in COVID-19 affected patients: a retrospective series. Clin Toxicol 2021; 59: 644-647.
27) Patel K, Lindsey J, Angelidou K, Aldrovandi G, Palumbo P; IMPAACT P1060 Study Team. Metabolic effects of initiating lopinavir/ritonavir-based regimens among young children. AIDS 2018; 32: 2327-2336.
28) Matoga MM, Hosseinipour MC, Aga E, Ribaudo HJ, Kumarasamy N, Bartlett J, Hughes MD; ACTG A5230 Study Team. Hyperlipidemia in HIV-infected patients on lopinavir/ritonavir monotherapy in resource-limited settings. Antivir Ther 2017; 22: 205-213.
29) Reyskens KM, Fisher TL, Schisler JC, O’Connor WG, Rogers AB, Willis MS. Cardio-metabolic effectsof HIV protease inhibitors (lopinavir/ritonavir). PLoS One 2013; 8: e73347.
30) Rajadurai M, Stanely Mainzen Prince P. Preventive effect of Naringin on lipids, lipoproteins and lipid metabolic enzymes in isoproterenol-induced myocardial infarction in Wistar rats. J Biochem Mol Toxicol 2006; 20: 191-197.
31) Jutrić D, Đikić D, Boroš A, Odeh D, Drozdek SD, Gračan R, Dragičević P, Crnić I, Jurčević IL. Effects of naringin and valproate interaction on liver steatosis and dyslipidaemia parameters in male C57BL6 mice. Arh Hig Rada Toksikol 2022; 73: 71-82.
32) Jeon SM, Park YB, Choi MS. Antihypercholesterolaemic property of Naringin alters plasma and tissue lipids,cholesterol-regulating enzymes, fecal sterol and tissue morphology in rabbits. Clin Nutr 2004; 23: 1025-1034.
33) Pistell PJ, Gupta S, Knight AG, Domingue M, Uranga RM, Ingram DK, Kheterpal I, Ruiz C, Keller JN, Bruce-Keller AJ. Metabolic and neurologic consequences of chronic lopinavir/ritonavir administration to C57BL/6 mice. Antiviral Res 2010; 88: 334-342.
34) Cavenaghi FM, Bataglion CAN, Paula PC, Motta ACF, Komesu MC. Protease inhibitor and metabolic alteration. Int J Morphol 2012; 30: 439-444.
35) Carpentier A, Patterson BW, Uffelman KD, Salit I, Lewis GF. Mechanism of highly active anti-retroviral therapy-induced hyperlipidemia in HIV-infected individuals. Atherosclerosis 2005; 178: 165-172.
36) Liang JS, Distler O, Cooper DA, Jamil H, Deckelbaum RJ, Ginsberg HN, Sturley SL. HIV protease

inhibitors protect apolipoprotein B from degradation by the proteasome: a potential mechanism for protease inhibitor-induced hyperlipidemia. Nat Med 2001; 7: 1327-1331.

37) Kim HJ, Oh GT, Park YB, Lee MK, Seo HJ, Choi MS. Naringin alters the cholesterol biosynthesis and antioxidant enzyme activities in LDL receptor-knockout mice under cholesterol fed condition. Life Sci 2004; 74: 1621-1634.

38) Zhang X, Zhang Y, Gao W, Guo Z, Wang K, Liu S, Duan Z, Chen Y. Naringin improves lipid metabolism in a tissue-engineered liver model of NAFLD and the underlying mechanisms. Life Sci 2021; 277: 119487.

39) Syed AA, Reza MI, Shafiq M, Kumariya S, Singh P, Husain A, Hanif K, Gayen JR. Naringin ameliorates type 2 diabetes mellitus-induced steatohepatitis by inhibiting RAGE/NF-κB mediated mitochondrial apoptosis. Life Sci 2020; 257: 118118.

40) Bonfili L, Cecarini V, Amici M, Cuccioloni M, Angeletti M, Keller JN, Eleuteri AM. Natural polyphenols as proteasome modulators and their role as anti-cancer compounds. FEBS J 2008; 275: 5512-5526.

41) Adikwu E, Nelson B, Obianime WA. Hepatic alterations in lopinavir/ritonavir–intoxicated rats were abrogated by melatonin and α-lipoic acid. J Anal Pharm Res 2019; 8: 112-117.

42) Cameron DW, Becker S, King MS, da Silva B, Klein C, Tokimoto D, Foit C, Calhoun D, Bernstein B, Hanna GJ. Exploratory study comparing the metabolic toxicities of a lopinavir/ritonavir plus saquinavir dual protease inhibitor regimen versus a lopinavir/ritonavir plus zidovudine/lamivudine nucleoside regimen. J Antimicrob Chemother 2007; 59: 957-963.

43) González-Requena D, Núñez M, Jiménez-Nacher I, González-Lahoz J, Soriano V. Short communication: liver toxicity of lopinavir-containing regimens in HIV-infected patients with or without hepatitis C coinfection. AIDS Res Hum Retroviruses 2004; 20: 698-700.

44) Seminari E, Gentilini G, Galli L, Hasson H, Danise A, Carini E, Dorigatti F, Soldarini A, Lazzarin A, Castagna A. Higher plasma lopinavir concentrations are associated with a moderate rise in cholestasis markers in HIV-infected patients. J Antimicrob Chemother 2005; 56: 790-792.

45) Mercer DW, Talamo TS. The role of biochemical markers in the management of cancer, In, Clinical Studies in Medical Biochemistry: Glew RH, Peters SP (ed). Oxford University Press 1987; 25-34.

46) Muragesh KS, Yeliogor VC, Maiti BC, Maity TK. Hepatoprotective and Antioxidenat role of Berberis tinetoria Lesch leaves on Paracetamol-induced hepatic damage in rats. Iranian J Pharmacol 2005; 22: 107-109.

47) Elias A, Oputiri D, Jimmy Z, Rejoice O, Marian A, Geoffrey OBP, Gilbert AT. Effect of co-administered lopinavir/ritonavir and sulfamethoxazole/trimethoprim on cardiac function and architecture of albino rats. Int J Clin Pharmacol 2017; 3: 817-823.

48) Adaramoye OA, Adewumi OM, Adesanoye OA, Faokunla O, Farombi EO. Effect of tenofovir, an anti-retroviral drug, on hepatic and renal functional indices of Wistar rats: protective role of vitamin E. J Basic Clin Physiol Pharmacol 2012; 23: 69-75.

49) Koroglu OF, Gunata M, Vardi N, Yildiz A, Ates B, Colak C, Tanriverdi LH, Parlakpinar H. Protective effects of Naringin on valproic acid-induced hepatotoxicity in rats. Tissue Cell 2021; 72: 101526.

50) Komulainen T, Lodge T, Hinttala R, Bolszak M, Pietilä M, Koivunen P, Hakkola J, Poulton J, Morten KJ, Uusimaa J. Sodium valproate induces mitochondrial respiration dysfunction in HepG2 in vitro cell model. Toxicology 2015; 331: 47-56.

51) Adil M, Kandhare AD, Ghosh P, Venkata S, Raygude KS, Bodhankar SL. Ameliorative effect of naringin in acetaminophen-induced hepatic and renal toxicity in laboratory rats: role of FXR and KIM-1. Ren Fail 2016; 38: 1007-1020.

52) Dong D, Xu L, Yin LH, Qi Y, Peng JY. Naringin prevents carbon tetrachloride-induced acute liver injury in mice. J Functional Foods 2015; 12: 179-191.

53) Kahle M, Schäfer A, Seelig A, Schultheiß J, Wu M, Aichler M, Leonhardt J, Rathkolb B, Rozman J, Sarioglu H, Hauck SM, Ueffing M, Wolf E, Kastenmueller G, Adamski J, Walch A, Hrabé de Angelis M, Neschen S. High fat diet-induced modifications in membrane lipid and mitochondrial-membrane protein signatures precede the development of hepatic insulin resistance in mice. Mol Metab 2014; 4: 39-50.

54) Merker H, Günther T, Höllriegl V, Vormann J, Schümann K. Lipid peroxidation and morphology of rat testis in magnesium deficiency. Andrologia 1996; 28: 43-51.

55) Vernet P, Aitken RJ, Drevet JR. Antioxidant strategies in the epididymis. Mol Cell Endocrinol 2004; 216: 31-39.

56) Farombi EO, Shrotriya S, Surh YJ. Kolaviron inhibits dimethyl nitrosamine-induced liver injury by suppressing COX-2 and iNOS expression via NF-kappaB and AP-1. Life Sci 2009; 84: 149-55.

57) Mostafa HES, Abd El-Baset SA, Kattaia AA, Zidan RA, Sadek A, Mona M. Efficacy of naringenin against permethrin-induced testicular toxicity in rats. Int J Exp Pathol 2016; 97: 37-49.

58) Ciz M, Denev P, Kratchanova M, Vasicek O, Ambrozova G, Lojek A. Flavonoids inhibit the respiratory burst of neutrophils in mammals. Oxid Med Cell Longev 2012; 2012: 181295.