Effects of Apnea, Obesity, and Statin Therapy on Proprotein Convertase Subtilisin/Kexin 9 Levels in Patients with Obstructive Sleep Apnea

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Abstract
Objectives: Obstructive sleep apnea (OSA) is a common condition closely related to obesity, insulin resistance, dyslipidemia, and cardiovascular disease. The aim of this study was to explore the possible relationship between OSA and proprotein convertase subtilisin/kexin type 9 (PCSK9). Methods: Full-night polysomnography was performed on 150 participants who were divided into three groups: controls, OSA patients on statin therapy, and OSA patients not on statin therapy. Biochemical markers, plasma low-density lipoprotein (LDL) and high-density lipoprotein (HDL) subclasses, and PCSK9 were determined. Results: PCSK9 was highest in OSA patients on statins compared to the control group and to OSA patients not on statins (p = 0.036 and p = 0.039, respectively), after adjustment for body mass index (BMI). LDL diameter was greater in OSA patients not on statins compared to OSA patients on statins (p = 0.032). PCSK9 was highest in the group of patients with all three risk factors (diagnosed OSA, statins, BMI ≥25 kg/m²) compared to groups with no, one, and two risk factors (p = 0.031, p = 0.001, and p = 0.029, respectively). Presence of OSA, statin therapy, and BMI ≥25 kg/m² when combined were independently associated with higher levels of PCSK9 when adjusted for antihypertensive therapy, small dense LDL, and HDL 3c subclass (odds ratio = 2.849; interquartile range [1.026–7.912], p = 0.044). Conclusion: Statin therapy was closely related to PCSK9. OSA along with obesity and statin use induces elevation of PCSK9.

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Introduction

Obstructive sleep apnea (OSA) is an increasingly prevalent condition characterized by repetitive episodes of complete decline of air flow (apnea) or partial decline of air flow (hypopnea) in the upper part of the respiratory system during sleep [1]. OSA develops in the presence of altered upper airway anatomy and defects in compensatory neuromuscular activities [1]. It is, however, closely associated with obesity, older age, and male gender [2]. Its clinical consequences include comorbidities such as type 2 diabetes [3], hypertension, stroke, coronary artery disease [4], and others. In recent decades, clinical studies have investigated the mechanisms by which OSA is related to these comorbidities. Although still not well understood, it is believed that the presence of chronic intermittent hypoxia (CIH) is a key factor [4]. CIH is associated with a vicious circle of oxidative stress, inflammation, and dyslipidemia, all of which underline the abovementioned atherosclerosis-related diseases [1, 4].

In general, dyslipidemia refers to elevated low-density lipoprotein cholesterol (LDL-C), high levels of triglycerides (TG), and low levels of high-density lipoprotein cholesterol (HDL-C) levels [5]. Advanced testing of lipid status has included lipoprotein particles characterization [6] and assessment of proprotein convertase subtilisin/kexin type 9 (PCSK9) [7]. PCSK9 is a serine protease synthesized by the liver, intestine, lung, kidney, and brain [7, 8] with its major role being to stimulate degradation of the hepatic LDL receptor (LDL-R) by binding to it and carrying the receptor-lipoprotein particle complex to lysosomes. Thus, the recirculation of LDL-R to the cell surface is suppressed [8]. This leads to lower expression of LDL-R on the cell membrane and increased levels of LDL-C.

Statin (a hydroxymethylglutaryl coenzyme A reductase inhibitor) treatment is the most widely used therapy which lowers cholesterol content in hepatic cells [9]. In clinical practice, PCSK9 inhibitors, together with statins, decrease cardiovascular risk in patients with stable atherosclerotic cardiovascular disease or recent acute coronary syndromes [10]. As a new biomarker and a very efficient target for LDL-C lowering therapy [11], the regulation of PCSK9 in OSA is still poorly understood.

We sought to determine whether the presence of OSA, alone or in conjunction with obesity and statin therapy, could influence circulating PCSK9 levels. Additionally, associations between the investigated parameters of obesity-related dyslipidemia and PCSK9 in OSA patients were examined.

Methods

Study Participants

The current cross-sectional study included subjects who were referred to the Pulmonology Department of the University Hospital Medical Center “Bežanijska kosa” for polysomnography examination from November 2016 to December 2017. The inclusion criteria were signed informed consent forms, age over 20 years, and clinical suspicion of OSA. The exclusion criteria were the presence of central apnea, inherited metabolic disorders, and long-term use of antidepressants and sedatives. On admission to hospital, all subjects completed a questionnaire about their family history, disease burden, and lifestyle habits. This was followed by height and weight measurements. Body mass index (BMI) was calculated. The study population consisted of 150 participants, of whom 34 belonged to the control group as their polysomnography results excluded OSA while 116 had proven OSA after polysomnographic testing. The 116 were divided into 2 groups, 94 OSA patients who were not on statin therapy and 22 OSA patients who were statin users. The subjects in the control group were not on statin therapy. None of the participants used insulin therapy. This study was conducted in agreement with the Helsinki Declaration and was approved by the University of Belgrade-Faculty of Pharmacy (No. 1073/2) and the University Hospital Medical Center “Bežanijska kosa” Ethics Committees (No. 3743/5).

Sleep Study

Full-night polysomnography studies were performed at the Pulmonology Department of the University Hospital Medical Center “Bežanijska kosa” using an Alice PDx device (Philips Respironics, Maineville, OH, USA). OSA severity was defined according to the apnea-hypopnea index (AHI). The AHI is the number of apneas and/or hypopneas per hour of sleep. Other measurements included minimum oxygen saturation (SaO2), average SaO2, and oxygen desaturation index (ODI). ODI is the number of times per hour that oxygen saturation drops ≥3% from baseline. Together, AHI <5 and ODI <5 were the main exclusion criteria for OSA diagnosis. An AHI >5 was considered as OSA. However, participants who had AHI 5-6 and ODI <2 were considered as not having OSA.

Biochemical Measurements

Peripheral venous blood samples were collected after full-night polysomnography and a 12-h fasting period. Serum concentrations of TG, total cholesterol (TC), and HDL-C were measured by routine methods on a Roche Cobas 6000 Analyzer (Roche Diagnostics, Basel, Switzerland). LDL-C concentration was calculated according to Friedewald’s equation.

Circulating plasma PCSK9 levels were quantified by ELISA (Quantikine ELISA, R&D Systems Europe Ltd, Abingdon, UK). Plasma LDL and HDL subclasses were separated using nondenaturing polyacrylamide gradient gel electrophoresis [12].

Statistical Analysis

The normality of groups containing less than 60 data points was checked with the Shapiro-Wilk test and normality of groups with more than 60 data points with the Kolmogorov-Smirnov test. Normally distributed data are presented as mean ± standard deviation, skewed variables as median (interquartile range), and categorical data are presented as relative and absolute frequencies. Data which achieved normality after logarithmic transformation are shown as

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geometrical mean (95% confidence interval, CI). Differences in demographic and cardiorespiratory markers between groups were assessed by one-way ANOVA with Tukey post hoc test, Kruskal Wallis, and Mann-Whitney tests. The χ² test for contingency tables was used for categorical data comparison purposes. As obesity affects lipid status, analysis of covariance and rank analysis of covariance (Quade’s test) were conducted in order to determine if a statistically significant difference existed between the tested groups using BMI as covariate. Associations between variables were identified by Spearman rank correlation testing. Binary logistic regression was used to examine whether OSA, statin therapy, and BMI were associated with higher PCSK9 levels. PCSK9 (the dependent variable) higher and equal to the 75th percentile of the control group was coded as 1 and that less than the 75th percentile was coded as 0. The independent variable was also categorical. Participants with no, one, or two risk factors were coded as 0. Participants with all three factors were stated as high risk and coded as 1. Multivariable binary regression analysis was conducted to examine possible independent association of the presence of all risk factors and PCSK9 level. Covariates were categorical data significantly different between the tested groups and data which correlated significantly with PCSK9 levels. Statistical analysis was performed using SPSS version 22.0 for Windows (SPSS, Chicago, IL, USA). A value of \( p < 0.05 \) was considered significant.

**Results**

**Anthropometric, Cardiopulmonary, and Laboratory Findings**

Basic demographic and clinical data of the tested groups are shown in Table 1. Older participants were among those with OSA regardless of statin therapy, compared to the control group. In addition, BMI was significantly higher in OSA patients receiving and not receiving statin therapy compared to the control group. There was a high prevalence of antihypertensive therapy users among OSA patients compared to the control group. Polysomnographic data were significantly higher in OSA patients regardless of statin therapy compared to the control group (Table 1). When adjusted for BMI, LDL diameter was lower in OSA patients with statin therapy compared to OSA patients not on statin therapy. PCSK9 was highest in OSA patients on statins compared to the two other groups (Table 2).

**Correlation Analysis of PCSK9 and OSA Markers with Other Laboratory Data**

Spearman correlation analysis was performed within each tested group (Table 3). In the control group, PCSK9 correlated negatively with both AHI and ODI, but positively with TC. No correlations were evident between PCSK9 and AHI with LDL and HDL particles. In OSA patients not receiving statin therapy, PCSK9 correlated positively with BMI, TC, LDL-C, the proportion of smaller LDL subfractions (LDL IV and small, dense LDL [sdLDL]), and the proportion of smaller HDL 3c particles. Negative correlation was significant only between PCSK9 and the proportion of larger LDL subfractions.
Table 2. Biochemical markers in the study groups

|                  | Control group | Patients without statin therapy | Patients on statin therapy | p value |
|------------------|---------------|---------------------------------|----------------------------|---------|
| TC, mmol/L       | 5.7±1.1       | 5.6±1.1                         | 5.4±1.1                    | 0.786   |
| TG, mmol/La      | 1.7 (1.5–1.9) | 1.7 (1.6–1.9)                   | 2.1 (1.7–2.4)              | 0.144   |
| LDL-C, mmol/Lb   | 3.7 (3.0–4.3) | 3.4 (2.9–3.9)                   | 3.0 (2.4–3.6)              | 0.151   |
| LDL diameter, nm | 26.2 (25.5–26.9) | 26.3 (25.5–27.1)                 | 25.4 (24.0–26.3)           | 0.032   |
| LDL I, %b        | 23.4 (20.4–31.2) | 22.4 (18.9–26.6)                 | 19.8 (17.9–21.8)           | 0.196   |
| LDL II, %b       | 30.0 (24.4–35.4) | 30.1 (25.1–36.24)                | 26.0 (23.5–34.8)           | 0.237   |
| LDL III, %b      | 19.8 (16.9–23.3) | 21.8 (17.6–23.6)                 | 23.0 (20.6–25.4)           | 0.088   |
| LDL IV, %b       | 21.5 (18.5–26.2) | 22.9 (17.5–32.2)                 | 27.5 (18.7–36.4)           | 0.375   |
| sdLDL, %b        | 42.7 (37.3–52.8) | 45.1 (37.7–55.2)                 | 53.2 (46.5–57.8)           | 0.090   |
| HDL-C, mmol/La   | 1.19 (1.10–1.30) | 1.19 (1.13–1.25)                 | 1.21 (1.09–1.33)           | 0.971   |
| HDL diameter, nm | 8.70 (8.44–9.77) | 8.87 (8.38–9.90)                 | 8.59 (8.16–9.83)           | 0.557   |
| HDL 2b, %b       | 31.0 (26.5–37.1) | 31.0 (27.1–38.4)                 | 29.6 (26.6–36.9)           | 0.549   |
| HDL 2a, %b       | 21.7 (19.4–25.7) | 20.9 (18.9–23.8)                 | 20.3 (17.9–22.9)           | 0.245   |
| HDL 3a, %b       | 20.7 (17.5–23.7) | 19.1 (16.8–21.2)                 | 18.8 (16.6–21.0)           | 0.085   |
| HDL 3b, %b       | 13.9 (9.5–16.2) | 12.9 (10.3–15.5)                 | 14.7 (11.8–17.5)           | 0.137   |
| HDL 3c, %b       | 9.9 (7.8–12.2) | 12.6 (8.1–17.2)                  | 15.3 (11.8–17.8)           | 0.153   |
| PCSK9, ng/mL     | 274.1±89.3    | 285.0±85.8                      | 335.9±86.2d,c              | 0.027   |

Data were adjusted for BMI and expressed as mean ± standard deviation. TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; sdLDL, small, dense low-density lipoprotein. a Log-transformed data are presented as adjusted geometrical mean (95% CI) and compared by ANCOVA after logarithmic transformation. b Data were presented as median (interquartile range) and compared by Quade’s test. c Significantly different from the group of patients with OSA without statin therapy (c: p < 0.05). d Significantly different from the control group (d: p < 0.05).

Table 3. Significant Spearman correlation of PCSK9 and AHI with other clinical markers

|                  | Control group | Patients without statin therapy | Patients on statin therapy | All participants |
|------------------|---------------|---------------------------------|----------------------------|-----------------|
| PCSK9, ng/mL     |               |                                 |                            |                 |
| AHI, n/h         |               |                                 |                            |                 |
| Age, years       | 0.062         | 0.119                           | −0.465c                    | 0.100           |
| BMI, kg/m²       | −0.465c       | 0.119                           | −0.118                      | 0.272c          |
| AHI, n/h         | −0.405a       | 0.361a                          | −0.249                      | 0.242c          |
| ODI, n/h         | −0.405a       | 0.361a                          | −0.249                      | 0.242c          |
| TC, mmol/L       | 0.211         | 0.211                           | 0.096                       | 0.087           |
| LDL-C, mmol/L    | 0.377         | 0.211                           | 0.096                       | 0.087           |
| LDL I, %         | 0.187         | 0.186                           | −0.012                      | 0.153           |
| LDL II, %        | 0.186         | 0.186                           | −0.012                      | 0.153           |
| LDL IV, %        | −0.282        | −0.282                          | −0.012                      | 0.153           |
| sdLDL, %         | −0.211        | −0.211                          | −0.012                      | 0.153           |
| HDL-C, mmol/L    | 0.022         | 0.022                           | 0.010                       | 0.074           |
| HDL diameter, nm | 0.122         | 0.122                           | −0.010                      | 0.159           |
| HDL 2a, %        | −0.142        | −0.142                          | −0.010                      | 0.159           |
| HDL 3a, %        | 0.188         | 0.188                           | −0.010                      | 0.159           |
| HDL 3b, %        | 0.169         | 0.169                           | −0.010                      | 0.159           |
| HDL 3c, %        | 0.145         | 0.145                           | −0.010                      | 0.159           |

BMI, body mass index; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; sdLDL, small, dense low-density lipoprotein; HDL-C, high-density lipoprotein cholesterol. Data are presented as correlation coefficient (ρ). a p < 0.05. b p < 0.001. c p < 0.01.
PCSK9 in Obstructive Sleep Apnea

AHI correlated positively with BMI and TG. AHI did not correlate neither with LDL nor HDL particles. In OSA patients on statin therapy, PCSK9 correlated positively only with BMI but negatively with HDL 3b particles. No correlations were evident between AHI and LDL and HDL particles, except for negative correlation between AHI and HDL diameter. AHI correlated positively with BMI and TG and negatively with age. For all participants, Spearman correlation analysis revealed significant positive correlation of PCSK9 with BMI, AHI, TC, TG, and relative proportions of LDL IV, sdLDL, and HDL 3c particles (Table 3). Significant negative correlations were demonstrated between PCSK9 and relative proportion of LDL II. Positive correlations of AHI were demonstrated with age, BMI, TG, proportion of smaller LDL subfractions (LDL IV and sdLDL), and proportion of smaller HDL 3c particles. Negative correlations between AHI were identified with larger LDL particles (LDL I and LDL II), HDL-C, and proportion of HDL 2a and 3a particles (Table 3).

Comparison of PCSK9 Levels between Groups with Different Numbers of Risk Factors

PCSK9 was compared between different groups: participants without OSA, no statins, and BMI <25 kg/m² versus participants with only one risk factor (OSA or statins or BMI ≥25 kg/m²) versus participants with any two risk factors (OSA, statins, BMI <25 kg/m² or OSA, BMI ≥25 kg/m², no statins or BMI ≥25 kg/m², statins, no OSA) versus participants with all three risk factors (OSA, statins, and BMI ≥25 kg/m²).

Fig. 1. PCSK9 in all participants according to presence of test factors. Data are presented as mean ± standard deviation and compared by one-way ANOVA. p = 0.002 for one-way ANOVA. 0 – participants without OSA, without statin therapy, and BMI <25 kg/m² (N = 10). 1 – participants with only one risk factor (OSA or statins or BMI ≥25 kg/m²) (N = 34). 2 – participants with any of two risk factors (OSA, statins, BMI <25 kg/m² or OSA, BMI ≥25 kg/m², no statins or BMI ≥25 kg/m², statins, no OSA) (N = 86). 3 – participants with all three risk factors (OSA, statins, and BMI ≥25 kg/m²) (N = 20).
and statins and BMI ≥25 kg/m²) (Fig. 1). There was no significant difference in PCSK9 between groups with no, one, or any two factors. However, PCSK9 was higher in all participants with all 3 risk factors (Fig. 1).

**Binary Logistic Regression for the Association of OSA, BMI, and Statin Therapy with PCSK9**

We then studied the associations of OSA and PCSK9 using binary logistic regression analysis. A PCSK9 cut-off of 320.49 ng/mL (75th percentile of the control group) was used for patients’ classification. The odds ratio after univariable binary logistic regression analysis was 3.375 with 95% confidence interval (1.281–8.894), \(p = 0.014\). When adjusted for antihypertensive therapy, sdLDL, and HDL3c, the odds ratio was 2.849 (1.026–7.912), \(p = 0.044\). OSA patients on statin therapy and BMI ≥25 kg/m² had almost 3 times higher risk of having PCSK9 above the 75th percentile of the control group than patients with any two risk factors, one risk factor, or no risk factors.

**Discussion**

In this cross-sectional study, OSA was identified as a condition closely associated with PCSK9. Although OSA alone was not an independent predictor for increased circulating PCSK9, OSA together with obesity and statin use may lead to elevated PCSK9.

PCSK9 has been identified as an important player in LDL particle metabolism [8]. PCSK9 inhibitors are already included in therapy for high levels of LDL-C that do not respond to other forms of therapy [11]. Also, they can be added to statin therapy to improve cardiovascular outcomes in patients with atherosclerotic cardiovascular disease [10]. The expression of PCSK9 in liver is regulated by cellular cholesterol in the same way as that of LDL-R [13]. Low physiological or statin-treatment depletion of cell cholesterol levels mobilizes sterol regulatory element binding protein-2 (SREBP-2) which binds to the PCSK9 promoter of the sterol regulatory element-1 (SRE-1) and activates transcription. Statin therapy is thus a double-edged sword, increasing LDL-Rs on the one hand while increasing PCSK9 on the other [9, 14]. Our results are in accordance with such studies. The highest levels of PCSK9 were found in OSA patients on statins compared to the other two groups (Table 2). Recent studies have examined the expression of the PCSK9 gene and whether its role in lipid metabolism could be influenced by different pathophysiological conditions such as diabetes, coronary artery disease, and obesity [15–17].

OSA is highly prevalent worldwide, especially among males [2]. Due to its relationship with obesity, as also confirmed by our study (Tables 2, 3), OSA represents a huge burden to public health [18]. Studies have published inconclusive data on the effects of the degree of apnea on the development of dyslipidemia [4, 11, 18]. Data on the newly implicated biomarker of dyslipidemia, PCSK9, is scarce. We found no significant differences in the basic lipid parameters between all tested groups after adjustment for BMI (Table 2). Neither apnea nor apnea with statin treatment had any influence on TC, HDL-C, LDL-C, and TG levels. However, our results revealed significant positive correlations of AHI and TG in all groups except the control group and a negative correlation with HDL-C in all participants (Table 3). Also, AHI and ODI negatively correlated with PCSK9 in the control group but positively correlated when all participants were grouped (Table 3).

Different mechanisms have been proposed for how the presence of OSA initiates disturbances in metabolic homeostasis. The most important of these is CIH [4] which enhances oxidative stress, systemic inflammation, and dyslipidemia [4, 19] through induction of synthesis of hypoxia-inducible factor-1 (HIF-1) in the liver, which activates the SREBP-1c transcriptional factor and consequently increases PCSK9 [13].

Hypoxia existed in our OSA patients (Table 1) indicating the potential involvement of oxidative stress in PCSK9 gene regulation. In a study by Ding and colleagues [20], reactive oxygen species appeared to induce PCSK9 expression through nuclear factor kappa B (NF-κB) activation in hepatocytes.

From published data and from data in our study, one question arises; does the presence of apnea, statins, and obesity (solely or in combination) affect PCSK9? Although their relationship positively correlated (only when all participants were grouped) (Table 3), apnea was not a sufficient condition for the elevation of plasma PCSK9 (Table 2). PCSK9 was higher in OSA patients without statin treatment than in the control group, but this difference did not reach statistical significance (Table 2). When considering the induction of PCSK9 gene by SREBP-1c and NF-κB, the presence of apnea cannot be ruled out as a condition that impacts PCSK9. Levels of PCSK9 were highest when apnea, BMI ≥25 kg/m², and statin therapy coexist (compared to one or two of risk factors) (Fig. 1). In addition, in combination with both statins and BMI ≥25 kg/m² OSA significantly and positively associated with PCSK9 in univariate binary regression analysis. Furthermore, the association remained sig-
significant after the adjustment for antihypertensive therapy, sdLDL, and HDL 3c.

Dyslipidemia includes analysis of advanced lipid profile markers, LDL and HDL subclasses, and their diameters [6, 12, 21]. The only significant difference was found for LDL particle diameter being smaller in OSA patients on statin therapy compared to OSA patients not on statin therapy (Table 2). This is not surprising as statin therapy causes the removal of larger (higher diameter) compared to smaller (smaller diameter) LDL particles [22]. In our study, statins demonstrated their effect in the presence of OSA (Table 2). A significant association between PCSK9 and lipoprotein subclasses has been reported earlier [16, 23]. In our patients, PCSK9 positively correlated with the proportion of smaller LDL and HDL particles in OSA patients not receiving statin therapy and in all participants (when grouped) but negatively correlated with smaller HDL particles in OSA patients receiving statin therapy (Table 3).

Although the interactions between apnea, obesity, and statin therapy on circulating PCSK9 are quite complex, our study is the first to indicate their combinatorial effects. However, there are a few limitations. Our study (with cross-sectional design) demonstrated only significant associations between the tested confounders and PCSK9; the causality between them cannot be inferred. A prospective study, with the inclusion of more participants, should help confirm our results. Finally, the participants within the control group were younger than the OSA patients.

Conclusions

PCSK9 was highest in OSA patients treated with statins when adjusted for BMI compared to both the control group and OSA patients not on statin therapy. Although OSA and statin therapy did not significantly influence the relative proportions of LDL and HDL particles, PCSK9 was positively associated with smaller LDL and HDL particle subclasses in OSA patients not receiving statin therapy and in all participants. The presence of OSA, statins, and BMI ≥25 kg/m² was independently positively associated with increased PCSK9 above the 75th percentile of the control group.

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Statement of Ethics

This study was conducted in agreement with Helsinki Declaration and was approved by the University of Belgrade-Faculty of Pharmacy (No. 1073/2) and the University Hospital “Bežanijska kosa” (No. 3743/5) Ethics Committees. All enrolled patients gave their written consent to be included in the study.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding publication of this article.

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Author Contributions

All authors contributed to the conception and design of this study. Conceptualization, data curation, and formal analysis was performed by Ana Ninić, Vesna Spasojević-Kalimanovska, Nataša Bogavac-Stanojević, Marija Zdravković, and Vojislav Radosavljević. Laboratory work was performed by Ana Milojević, Lidija Memon, Jelena Vekić, Aleksandra Željković, Aleksandra Stefanović, Marija Mihajlović, and Jasmina Ivanšević. Patient enrollment was performed by Milica Brajković, Vera Gardijan, Marija Zdravković, and Vojislav Radosavljević. The first manuscript draft was prepared by Ana Ninić and Ana Milojević. Review and editing was performed by Vesna Spasojević-Kalimanovska, Nataša Bogavac-Stanojević, Jelena Vekić, Aleksandra Željković, Marija Zdravković, and Aleksandra Stefanović. All authors read and approved the final version of the manuscript.

Data Availability Statement

Data are available upon receipt of a reasonable request to aninic@pharmacy.bg.ac.rs.

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