ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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THE CURATIVE ROLE OF LINALOOL IN RIFAMPICIN-INDUCED BRAIN TISSUE DAMAGE

RİFAMPİSİN KAYNAKLI BEYİN DOKUSU HASARINDA LİNALOOL'UN İYİLEŞTİRİCİ RÖLÜ

Meltem ÖZGÖÇMEN[1], Sebile AZIRAK[2]

[1] Süleyman Demirel Üniversitesi, Tıp Fakültesi, Histoloji ve Embriyoloji Ana Bilim Dalı, Isparta, TÜRKİYE

[2] Adıyaman Üniversitesi, Sağlık Hizmetleri Meslek Yüksekokulu, Adıyaman, TÜRKİYE

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Öz

Amaç

Bu çalışmada antihiperglisemik, hipolipidemik ve antioksidan özelliklere sahip olan linalool (LN), rifampisine (RF) bağlı olarak oluşabilen nörodejenerasyonlar ve nöral bozuklukların tedavisi amacıyla kullanılmıştır. Bu amaçla, RF'ye bağlı oluşabilecek nörotoksisitede, antioksidan özelliği olan LN'nin koruyucu etkinliği hem gen ekspresyonu seviyesinde hem de biyokimyasal ve histopatolojik bulgularla araştırılmıştır.

Gereç ve Yöntem

30 adet sağlıklı erkek Spraque-Dawley rat beş gruba ayrıldı (Grup 1: Kontrol grubu; Grup 2: Çözücü Kontrol (Dimetilsülfoksit-DMSO) grubu; Grup 3: Rifampisin (RF) grubu; Grup 4: Linalool (LN) grubu; Grup 5: Rifampisin-Linalool (RF+LN) grubu. Biyokimyasal ve histolojik analizler ile gen ekspresyon seviyeleri için beyin dokuları, kan glikoz seviyelerini ölçmek için kan örnekleri alındı.

Bulgular

Rifampisin uygulaması, beyin ağırlığını azaltırken, CYP1A1 ve CYP1A2 mRNA gen ekspresyonunu ve kan glukoz düzeylerini önemli ölçüde artırmıştır. Diğer yandan RF+LN grubunda CYP1A1 ve CYP1A2 mRNA gen ekspresyonu ve kan glukoz düzeylerinde anlamlı azalmalar olurken, beyin ağırlığında anlamlı düzelme gözlenmiştir. Histolojik bulgular LN verilen gruplarda, RF ye bağlı oluşan hasarların azaldığını göstermiştir.

Sonuç

LN'nin beyin dokusunu RF'nin toksik etkilerinden korumada oldukça etkili olduğu tespit edilmiştir.

Anahtar Kelimeler: Beyin; CYP1A1, CYP1A2, Linalool, Rifampisin

Abstract

Objective

In this study linalool (LN), which has antihyperglycemic, hypolipidemic and antioxidant properties, is intended to be used in the treatment of neurodegenerations and neural disorders that may occur due to rifampicin (RF). For this reason, it was aimed to examine the effects of LN on the expression of genes, biochemical and histopathological parameters in these metabolic pathways against neurotoxicity that may occur due to RF, and to investigate the protective effects of LN, which has antioxidant properties.

Material and Method

Thirty healthy male Spraque-Dawley rats were divided into five groups (group 1; control, group 2; solvent

Sorumlu yazar ve iletişim adresi / Corresponding author and contact address: M.Ö. / meltemozgocmen@sdu.edu.tr
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ORCID IDs of the authors:M.Ö: 0000-0003-3190-4486; S.A: 0000-0001-9040-6773

control (DMSO); group 3, RF; group 4, LN; group 5; RF+LN). Brain tissues were taken for biochemical, histological and gene expressions analyses. Blood samples were taken to measure blood glucose levels.

Results
Rifampicin treatment significantly increased CYP1A1 and CYP1A2 mRNA gene expression and blood glucose levels, while reducing brain weight according to findings. On the other hand, there was a significant decrease in CYP1A1 and CYP1A2 mRNA gene expression and blood glucose levels in the RF+LN group, while a significant improvement in brain weight was observed and as a result of histological analyzes, it was observed that the damage caused by RF decreased in the groups given LN.

Conclusion
LN was found to be highly effective in protecting the brain from the toxic effects of RF.

Keywords: Brain, CYP1A1, CYP1A2, Linalool, Rifampicin.

Introduction

Rifampicin (RF) or rifampin is an antibiotic drug from the rifamycin group with a bactericidal effect. It is generally used in the treatment of mycobacterial infections (tuberculosis, leprosy, etc.) (1).

Rifampicin has some side effects. A 56-year-old patient developed bilateral retrobulbar neuritis that progressed to sensory polyneuropathy and severe optic atrophy 3 months after initiation of an antituberculosis regimen with isoniacid, ethambutol, and rifampicin (2). Widely used antibacterial agents such as RF have been associated with various neurotoxic reactions such seizure disorders, encephalopathy, as central nervous system toxicities, neuropsychiatric symptoms and swollen fontanelles (3).

CYP1A1 and CYP1A2 are two of the individual members of cytochrome P450 (CYP), one of the most important enzyme groups involved in drug metabolism (4). CYP1A1/1A2 *enzymes have been shown to play a key role in the expression of all genes*. CYPs are a super family of enzymes that catalyze the metabolic activation and detoxification of a wide variety of xenobiotics, including clinically used drugs, drugs of abuse, toxins (5). In addition, CYPs are involved in the metabolism of various endogenous chemicals such as bile acids, hormones, neurosteroids, eicosanoids, cholesterol, and vitamins (6).

The genes encoding cytochrome P450 are regulated by endogenous hormones such as growth hormone, corticosteroids, thyroid and sex hormones secreted from the hypothalamus-pituitary (7). Therefore, it has been reported that brain CYPs play an important role in the control of personality and behavior, brain activity, sensitivity and neuronal disorders. Members of the CYP family most active in the metabolism of toxic and chemical carcinogens include CYP1A1, 1A2, 1B1 and 2E1(8, 9).

The mammalian brain depends on glucose as its main energy source, and glucose metabolism is critical to brain physiology and function. Insulin secreted by the pancreas plays a major role in the regulation of glucose metabolism in peripheral tissues. The brain is an insulin sensitive organ and insulin has been shown to protect neurons against neurodegenerative disorders. Impaired insulin signaling causes oxidative neuronal damage and puts the brain at risk of neurodegeneration (10).

Oxidative stress through the production of reactive oxygen species (ROS) has been suggested as the main cause underlying the development of insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus (T2DM) and β-cell dysfunction (11, 12). Hyperglycemia triggers several metabolic signaling pathways that lead to inflammation, cytokine secretion, cell death and thus diabetic complications. Diabetic peripheral neuropathy (DPN) results from hyperglycemia that induces inflammatory responses and oxidative stress that damage nerve tissue (13). Hyperglycemia is also known to increase ROS production and cause oxidative stress. The increase in the production of ROS and the decrease in antioxidant capacity increase the inflammatory response and apoptosis (14).

Medicinal and aromatic plants are used for antidiabetes, anticancer, antihypertension, anticardiovascular, antileprosy, etc., due to their ability to synthesize therapeutic bioactive secondary metabolites has pharmacological properties. Although there are no treatment strategies to reduce oxidative stress in general, antioxidants are thought to be effective in this regard (15, 16). In our study, we used linalool (LN), a natural monoterpene, to eliminate or reduce the common side effects of RF.

Linalool has antioxidant activity, and antimicrobial, anxiolytic, antiviral, antidepressive, anticancer, anti-inflammatory, antinoceptive, anti-hyperlipidemic, analgesic, anxiolytic, and neuroprotective effects. LN has powerful antioxidant properties and eliminates ROS produced by various toxic substances (17).

Studies have shown that LN has neuroprotective effects against cortical neuronal damage induced by oxygen-glucose deprivation/reoxygenation (OGD/R) (18). However, the protective and therapeutic effects of LN in combination with RF on the brain have not been studied. In this study, it was aimed to investigate the effects of LN by giving RF to rats with gene expression, biochemical and histopathological analysis.

Material and Method

Ethical Approval
All experiments in this study were approved by Adıyaman University Animal Experiments Ethics Committee (2021/026).

Chemicals
Rifampicin (CAS RN: 13292-46-1) were purchased from TCI (Tokyo Chemical Industry) Chemicals. Linalool and all other chemicals used in the study were purchased from Sigma Aldrich.

Animals and Experimental Design
The study was carried out on a total of 30 healthy, 8-12 weeks old male Sprague Dawley rats, with an average weight of 220-270 g, Rats were housed at 21 ± 2 °C, 45% ± 5% humidity, and a 12:12 h light: dark cycle in a well-ventilated room. Free access to feed and water was provided. The rats were randomly divided into 5 equal groups according to the inducing and protective agents given to the rats. The first was used as a control. The second group, solvent control, 50 mg/kg/day Dimethyl sulfoxide (DMSO) was applied. The third group was given 50 mg/kg/day of rifampicin (19). The fourth group was given linalool 50 mg/kg/day (20). The fifth group was given linalool 50 mg/kg/day (20) followed by rifampicin 50 mg/kg/day.

Preparation of Test Solutions
Rifampicin (50 mg/kg/day) was prepared by dissolving in dimethyl sulfoxide (DMSO, Sigma, USA) and stored at -20 °C (21, 22). Linalool was administered alone as 50 mg/kg/day.

Treatments of Test Solutions
Except for the intraperitoneal application of linalool, all applications were administered daily by oral gavage for 15 days.

Measurement of Blood Glucose Level
Blood glucose levels were measured with Abbott Labs Architect C16000 systems (Abbott GmbH & Co, Germany) and the commercial Abbott kits were utilized. Blood glucose levels were determined by spectrophotometric method in the wavelength range of 340-380 nm (23, 24). Glucose levels were expressed as mg/dl serum.

Genetic Analysis
RNA Extraction, cDNA Synthesis and Quantitative PCR Analysis
Fresh frozen rat brain was processed for RNA extraction using AccuZol™ Total RNA Extraction Solution (Bioneer, K-3090) under RNAse-free conditions following the manufacturer's instructions and quantified by measuring absorbance at 260/230 nm and 260/280 nm with the NanoDrop spectrophotometer (Denovix DS). -11) and stored at -80 °C until use. For Q-PCR, the first 5 µg of total RNA was reverse transcribed using AccuPower® RT PreMix (Bioneer K-2041) using appropriate controls to ensure no genomic DNA contamination.

qRT-PCR was used to detect the expression level of CYP1A1 and CYP1A2 and was performed using the ExiCyclerTM96 qRT-PCR system (Bioneer) following the instructions of AccuPower GreenStar qPCR PreMix (Bioneer, Cat No: K-6210). The GAPDH gene was amplified as an internal control. The primers were for CYP1A1 forward, 5'- ATGAGTTTGGGGAGGTTACTGGT -3' and reverse, 5'- ACTTCTTATTCAAGTCCTTGAAGGCA-3' (25), CYP1A2 forward 5'- AGTCCAGGAACACTATCAAGACTTCA-3' and reverse 5'- AGGGATGAGACCACCGTTGTC-3' (25). GAPDH forward, 5'- CAACTCCCTCAAGATTGTCAGCAA-3' and reverse, 5'-GGCATGGACTGTGGTCATGA-3' (23). The PCR conditions were: 95 °C for 1 minute, then 45 cycles at 95 °C for 5 seconds and 55 °C for 40 seconds. The 2^-ΔΔCt method was used to calculate the relative mRNA expression.

Histological Procedure
The brain tissues were fixed in 10% formalin solution for 24 hours and washed in tap water one night after fixation. Tissue samples were dehydrated in through increasing concentrations of ethanol, cleared with xylene, and embedded in paraffin. The sections at 4 µm thickness taken from paraffin blocksby using microtome (Leica SM2000R, Germany) were stained by Hematoxylin–Eosin (H–E), and then covered with entellan.

Immunohistochemical Procedure
Tissues of 4µm thicknesses were obtained and were stained with CAS-8 primary ab (rabbit Caspase 8 antibody, ab25901, Abcam, Cambridge, USA, 1:100) and iNOS primary ab (rabbit anti iNOS antibody, ab3523 Abcam, Cambridge, USA, 1:100) by using a sensitive peroxidase-labelled streptavidin-biotin detection system (B40931, Abcam, Cambridge, USA) and were covered with entellan. At last, tissue samples were analysed and evaluated, the receptor densities observed were identified using the semi-quantitative evaluation method (26).

Histological Evaluation
All specimens were examined under light microscope (Nikon Eclipse Ni-U, JAPAN) and were photographed by digital camera (Nikon DS-Ri2, JAPAN). On the specimens stained with Hematoxylin–Eosin, a semi-quantitative analysis of histopathological results were then calculated to allow comparison between the groups. All groups were analysed and evaluated according to scoring by Refaiy (26).

Histopathological findings were graded as follows:

0 (-), negative score: No structural changes,

1 (+), 1 positive score: Light structural changes,

2 (++), 2 positive score: Middle structural changes,

3 (+++), 3 positive score: Serious structural changes

Immunohistochemical Evaluation
The staining intensity were identified using the semi-quantitative evaluation method on immunuhistochemically staining sections (26). Samples were analyzed by examining five different sections in each sample, which were then scored from 0 to 3 (-, +, ++, +++) according to the intensity of staining.

Immunoreactivity scores were graded as follows:

0 (-), negative score: absence of immunopositive reaction,

1 (+), 1 positive score: slight immunopositive reaction,

2 (++), 2 positive score: moderate immunopositive reaction,

3 (+++), 3 positive score: strong immunopositive reaction.

For evaluation, 10 different fields for every section at a magnification under 20X objective for all groups. Results were subjected to statistical analysis.

Statistical Analysis
Data were analyzed and Mann-Whitney U and one-way ANOVA test were conducted as described in (27) via SPSS 18 software for all analysis. Differences were considered as significant for $P<0.05$. The values were expressed as mean ± standard deviation (S.D), as appropriate, and at least in triplicate.

Results

Effects of RF and LN on Fresh Brain Weight (g) and Fresh Brain Weight/Body Weight Ratio
Brain weight measurements were shown in Table 1. It is seen that the brain weight of the animals treated with RF decreased and this decrease was statistically significant compared to the control, LN, RF+LN groups (p=0.000). On the contrary, a statistically significant increase was found in the brain weight of the

Table 1 Comparison of fresh brain weight (g) and fresh brain weight/body weight ratio between the groups.

	STUDY GROUPS					
	Control	**DMSO**	**RF**	**LN**	**RF+LN**	**p value**
Fresh brain weight (g)	2,01±0,0038 ^{c,e}	1,9796±0,0033^{c,e}	1,7266±0,0112^{a,b,d,e}	1,9851±0,0030^{c,e}	1,8678±0,0187^{a,b,c,d}	0,000
Fresh brain weight/body weight ratio	0,0065±0,00003$^{b,\ c,e}$	0,0065±0,00002 ^{c,e}	0,0061±0,00003 ^{a,b,d,e}	0,0065±0,00002^{c,e}	0,0066±0,00003 ^{a,b,c,d}	0,000

Each group represents the mean ± SEM for experimental rats. The was used ANOVA test a; Significant from control; b; Significant from DMSO; c;Significant from RF; d; Significant from LN; e; Significant from RF+LN. p<0.05. Abbreviations: RF: rifampicin 50 mg/kg/day; DMSO: dimethyl sulfoxide; LN: linalool 50 mg/kg/day RF+LN: 50 mg/kg/day rifampicin+50 mg/kg/day linalool.

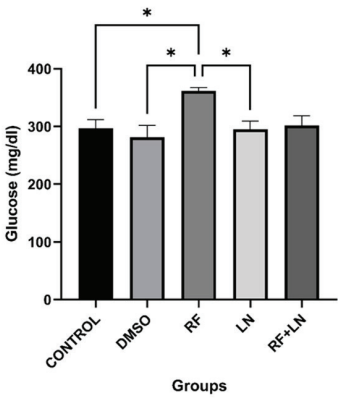

Figure 1
Effect of RF and LN on blood glucose level in rats in a 15-day experiment. Each group represents the mean ± SEM for six rats. The was used one-way ANOVA with Graphpad 9 Prism. Data are the mean ± SEM of six individual rats. * p<0.05. Abbreviations: RF: rifampicin 50 mg/kg/day; DMSO: dimethyl sulfoxide; LN: linalool 50 mg/kg/day RF+LN: 50 mg/kg/day rifampicin+50 mg/kg/day linalool.

RF+LN group compared to the RF group (p=0.000) (Table 1). In addition, the fresh brain weight/body weight ratio was statistically different between all groups (p=0.000) (Table 1). According to results, LN showed preventive and ameliorative effect against the common side effects of RF.

Biochemical Results

Effects of RF and LN on Blood Glucose Levels

The effects of RF and LN on blood glucose levels are summarized in Figure 2. Significant differences were detected between the various treatments for glucose levels in rats. In RF group rats, blood glucose levels had statistically significantly higher compared to control, DMSO and LN group rats (p<0.05). However, although blood glucose values were low in the RF+LN group than the RF group, it was not statistically significant (p=0.0768, p>0.05).

Genetic Results

Effect of RF and LN on Expressions of CYP1A1 and CYP1A2 Gene

Figures 2A and 2B show the effects of RF versus LN on CYP1A1 and CYP1A2 mRNA expression in all study groups. CYP1A1 (p<0.0001) and CYP1A2 (p<0.01) mRNA expressions were significantly increased in the RF group compared to the control, DMSO, LN and RF+LN groups. The RF+LN group significantly reduced CYP1A1 (p<0.0001) and CYP1A2 (p<0.01) mRNA levels compared to the RF group (Fig. 2A and 2B).

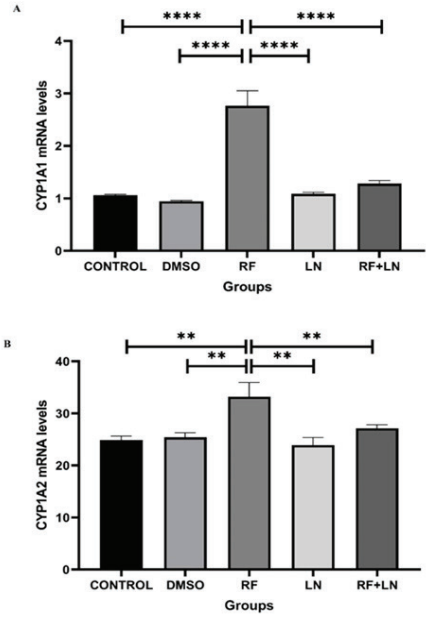

Figure 2
Effect of RF and LN on expressions of CYP1A1 (A) and CYP1A2 (B) mRNA in rat brain. Results were analyzed by real-time PCR and the values obtained were normalized against the expression of the endogenous control GAPDH. The were used ANOVA with Graphpad 9 Prism. Data are the mean ± SEM of six individual rats. **** p<0.0001, ** p<0.01. Abbreviations: RF: rifampicin 50 mg/kg/day; DMSO: dimethyl sulfoxide; LN: linalool 50 mg/kg/day RF+LN: 50 mg/kg/day rifampicin+50 mg/kg/day linalool.

Histological Findings

Normal histological structures were observed in the control, DMSO and LN groups (Figs. 3 A, B, and D). Histopathological findings were observed in RF and RF+LN groups such as mononuclear cell infiltration, neuropil vacuolization, neuron degeneration, picnotic nucleus in neurons and hemorrhagic areas, decrease in the number of neurons (p<0.05), (Figs. 3 Cand E). Mentioned tissue damages were decreased in RF+LN group compared to RF group (p<0.05), (Fig. 3 E).

Immunohistochemical Results

It was observed that the CAS-8 and iNOS receptors in the brain sections were stained very slightly in the control, DMSO and LN groups (Figs. 4A, A1, B, B1, D, D1), (Table 2). On the other hand, they were more intensely stained in the RF and RF+LN groups compared to the control, DMSO and LN groups (p<0.05), (Figs. 4C, C1, 4E, E1), (Table 2).

The staining intensity of the receptors is highest in RF and RF+LN groups, respectively, while less in control, DMSO and LN groups (p<0.05), (Fig. 4), (Table 2).

Table 2 The mean immunoreactivity scores for iNOS and Cas-8positive cells in brain tissues of the groups.

	Control	DMSO	RF	LN	RF+LN
iNOS	0.13±0.344^{c,e}	0,28±0,254^{c,e}	2,88±0,154^{a,b,d,e}	0.18±0.363^{c,e}	1,68±0,154^{a,b,c,d}
Cas -8	0.15±0.363^{c,e}	0,13±0,62^{c,e}	2,15±0,263^{a,b,d,e}	0.13±0.254^{c,e}	1,42±0,254^{a,b,c,d}

Values are expressed as means ± SD. The relationships between groups and results are assessed by one-way ANOVA. a, b, c, d, e; different characters indicate statistically significant differences in the same column $p<0.05$, $p<0.001$. a; Significant from control; b; Significant from DMSO; c;Significant from RF; d; Significant from LN; e; Significant from RF+LN.

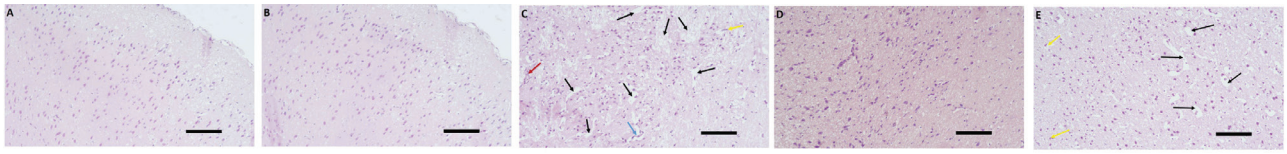

Figure 3
Histopathological findings in brain tissues belonging to control and experimental groups: A, B, D; Control, DMSO and LN groups; normal histological structure of brain. C, RF group; picnotic nukleus in neurons (yellow arrow), mononuclear cell infiltration (red arrow), neuropil vacuolization (black arrowss), neuron degeneration (blue arrowss). E, RF+LN group; mild histopatological findings, H-E, x20.

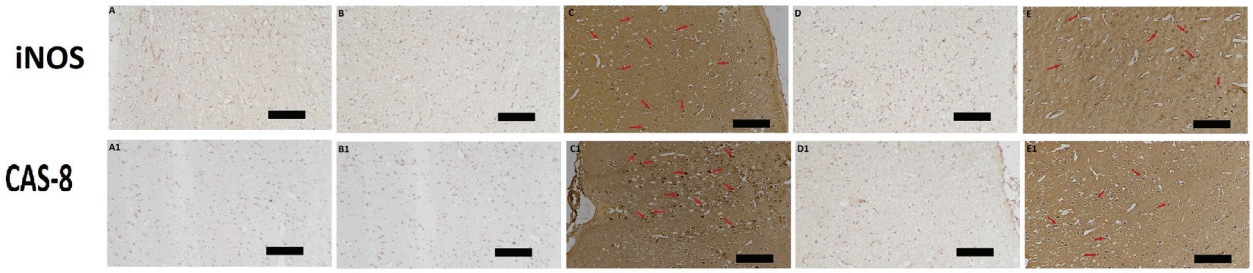

Figure 4
iNOS and CAS-8 immune stainings in brain tissues belonging to control and experimental groups. A-A1, B-B1, D-D1; Control, DMSO and LN groups; no positive staining, C-C1, RF group; intensive positive staining, E-E1, RF+LN group; mild positive stainings, A-B-C-D-E; iNOS, A1-B1-C1-D1-E1; Caspase 8, immün satining, x20.

iNOS immunoreactivity was stronger than CAS-8 for RF and RF+LN groups ($p<0.05$), (Fig. 4 C, C1, 4E, E1).

Discussion

In recent years, several studies have demonstrated the presence of drug-metabolizing enzymes, particularly cytochrome P450s, in all brain regions of different species and in various brain cells (28). The possibility of CYP1A1/1A2 being localized and inducible at the brain-CSF barrier and in leaky areas of the blood brain barrier where they may act as a protective metabolic barrier was examined (29).

In present study, rifampicin (RF) was metabolized by the cytochrome P450 (CYP) dependent monooxygenase system. RF induced CYP1A1 and CYP1A2. In a previous study, cells that differentiate into stem cells transformed into neuronal subtypes showed significant induction in the expression of AHR, CAR,

PXR, CYP1A1, 2B6, 2E1, 3A4 and GSTP1-1 when exposed to the universal CYP inducer RF, supporting the results obtained from this study. (30). Abnormalities in the brain such as neurodegeneration, incidence of dementia and mild cognitive impairment occur due to neuronal redox imbalance and oxidative damage (31).

According to present findings, was found that the brain weights and also the ratio of brain/body weight of rats treated with RF decreased. It was thought that this situation is due to cytotoxicity and apoptosis that develops increased CYP1A1/1A2 expression in the brain caused by RF. In a previous study, showing increased cytotoxicity, micronuclei formation, caspase 3/7 activity, apoptosis and DNA damage in cells due to increased expression of CYP1A1/1A2 provides consistent evidence supporting this findings (32). In another study, it was reported that increased CYP1A1/1A2 expression causes apoptosis and cell damage by increasing oxidative stress (33). Studies have shown that bioactive and unstable intermediate metabolites are produced by the catalytic effect of CYP enzymes from ubiquitous environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs), and these metabolites are cytotoxic and mutagenic and are generally detoxified by the enzymatic effect of phase II enzymes. (34). Studies have found a positive relationship between stimulation of CYPs and oxidative stress (35). In the studies, it was observed that the formation of DNA adducts increased as a result of the induction of the CYP1A1 enzyme (36). In a study, it was reported that rifampicin can cross the blood-brain barrier and cause obstruction in the meninges (37). These enzymes are thought to be involved in various central nervous system functions, especially in the protection of brain tissue from toxic substances, neurosteroid synthesis and drug metabolism (38). In this study, similar findings were observed with genetic results.

According to the results obtained from the present study, it was thought that LN protects brain tissues from apoptosis and cytotoxicity, especially by reducing CYP1A1/1A2 mRNA expression against the side effects of RF, and LN achieves this effect by eliminating ROS caused by CYP1A1/1A2 expressions, thanks to its antioxidant property. In a study, treatment with LN showed attenuation of cancer activity by inhibiting mRNA expression of proapoptotic markers such as BcL-2, Cas-3, CYP1A1 and NFκB (39). Caputo et al (2021) found that LN prevented neurotoxicity by inhibiting the increase in the production of intracellular ROS and the activation of caspase-3 (40). In studies was found that apoptotic pathway was caused by the intrinsic mitochondrial pathway with the decreased cleaved caspases (3-8) (41-43). Similar findings were also observed immunohistochemically with staining iNOS and Cas-8 in this study. The higher degree of iNOS staining than Cas-8 staining, indicates that apoptosis occurred due to the increase in NO.

In an another study, conducted on diabetic rats, consumption of cinnamon, the active ingredient of which is LN, significantly reduced blood glucose, triglyceride, total cholesterol, VLDL, LDL and urea levels, while on the contrary increased GSH activity (44). Similar findings were observed in this study as well.

The brain is a glucose-dependent organ that can be damaged by both hypoglycemia and hyperglycemia, and has a key role in the control of energy metabolism, glucose metabolism and body fat content (45). In present study, blood glucose levels increased significantly in rats treated with RF, while treatment with LN improved blood glucose levels. The study, which reported that plasma glucose and insulin increased by 192% and 45%, respectively, when 600 mg of RF was administered to the subjects once a day for 7 days supports our findings (46). It has been suggested that elevated glucose levels in diabetes mellitus with RF have deleterious effects on cells through the lysosomal mechanism (47). Deepa et al shown that LN treatment restored glucose metabolizing enzymes, collagen content and GLUT-1 expression and protect from oxidative stress and inflammation in diabetic rats (48).

In present study, high blood glucose level caused apoptosis and cytotoxicity by increasing ROS in the brain. A study showing that high glucose levels cause an increase in ROS in the brain and neuronal apoptosis by increasing both oxygenase-1 (HO-1) expression from astrocytes supports this study's findings (49). Niemi et al have reported that RF reduces the blood sugar-lowering effect of glimepiride, an antidiabetic drug. (50). Therefore, blood glucose levels should be closely monitored, especially if RF is added to or removed from the treatment of a diabetic patient. Studies have shown that 1-week treatment with rifampicin impairs glucose tolerance in healthy volunteers (46).

Conclusion

Studies show that there is a lack of literature on the expression and inducibility of xenobiotic metabolizing CYPs in the brain. For this purpose, in this study, it was determined that the ameliorative and preventive effects of LN on CYP1A1 and CYP1A2 expressions, brain weights and blood glucose levels in the brain tissues of RF administered rats. However, further clinical studies are needed on this subject.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
All experiments in this study were approved by Adıyaman University Animal Experiments Ethics Committee and were performed according to ethical rules (2021/026) and the researchers paid great attention to the welfare of animals during the study.

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Availability of Data and Materials
All data and materials are available in the article and/or supplementary files.

Authors Contributions
M.Ö: Investigation, Histological-Immunohistological analyzes, Data curation, Validation, Visualization, Writing.

S.A: Investigation, Data curation, Validation, Visualization, Writing.

References

1. Chiba S, Tsuchiya K, Sakashita H, Ito E, Inase N. Rifampicin-induced acute kidney injury during the initial treatment for pulmonary tuberculosis: a case report and literature review. Internal medicine. 2013;52(21):2457-60.
2. Leppert D, Waespe W. Neurotoxicity of antituberculous drugs in a patient without active tuberculosis. Italian journal of neurological sciences. 1988 Feb;9(1):31-4.
3. Snavely SR, Hodges GR. The neurotoxicity of antibacterial agents. Annals of internal medicine. 1984 Jul;101(1):92-104.
4. Hiratsuka M. In vitro assessment of the allelic variants of cytochrome P450. Drug metabolism and pharmacokinetics. 2012;27(1):68-84.
5. Ferguson CS, Tyndale RF. Cytochrome P450 enzymes in the brain: emerging evidence of biological significance. Trends in pharmacological sciences. 2011 Dec;32(12):708-14.
6. Liu M, Hurn PD, Alkayed NJ. Cytochrome P450 in neurological disease. Current drug metabolism. 2004 Jun;5(3):225-34.
7. Rysz M, Bromek E, Haduch A, Sadakierska-Chudy A, Daniel WA. Damage to the Brain Serotonergic System Increases the Expression of Liver Cytochrome P450. Drug metabolism and disposition: the biological fate of chemicals. 2015 Sep;43(9):1345-52.
8. Kim JH, Stansbury KH, Walker NJ, Trush MA, Strickland PT, Sutter TR. Metabolism of benzo[a]pyrene and benzo[a]pyrene-7,8-diol by human cytochrome P450 1B1. Carcinogenesis. 1998 Oct;19(10):1847-53.
9. Ioannides C, Lewis DF. Cytochromes P450 in the bioactivation of chemicals. Current topics in medicinal chemistry. 2004;4(16):1767-88.
10. Muller AP, Haas CB, Camacho-Pereira J, Brochier AW, Gnoatto J, Zimmer ER, et al. Insulin prevents mitochondrial generation of H_2O_2 in rat brain. Experimental neurology. 2013 Sep;247:66-72.
11. Wright E, Scism-Bacon JL, Glass LC. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. International journal of clinical practice. 2006 Mar;60(3):308-14.
12. Yang H, Jin X, Kei Lam CW, Yan SK. Oxidative stress and diabetes mellitus. Clinical chemistry and laboratory medicine. 2011 Nov;49(11):1773-82.
13. Cheng YC, Chu LW, Chen JY, Hsieh SL, Chang YC, Dai ZK, Wu BN. Loganin Attenuates High Glucose-Induced Schwann Cells Pyroptosis by Inhibiting ROS Generation and NLRP3 Inflammasome Activation. Cells. 2020 Aug 23;9(9):1948. doi: 10.3390/cells9091948.
14. Rizwan H, Pal S, Sabnam S, Pal A. High glucose augments ROS generation regulates mitochondrial dysfunction and apoptosis via stress signalling cascades in keratinocytes. Life sciences. 2020;241:117148.
15. Ahmad KA, Yuan Yuan D, Nawaz W, Ze H, Zhuo CX, Talal B, et al. Antioxidant therapy for management of oxidative stress induced hypertension. Free radical research. 2017 Apr;51(4):428-38.
16. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. European journal of medicinal chemistry. 2015 Jun 5;97:55-74.
17. Wang W, Li N, Luo M, Zu Y, Efferth T. Antibacterial activity and anticancer activity of Rosmarinus officinalis L. essential oil compared to that of its main components. Molecules. 2012 Mar 5;17(3):2704-13.
18. Park H, Seol GH, Ryu S, Choi IY. Neuroprotective effects of (-)-linalool against oxygen-glucose deprivation-induced neuronal injury. Archives of pharmacal research. 2016;39(4):555-64.
19. Hussain T, Subaiea GM, Firdous H. Hepatoprotective Evaluation of Trapa natans against Drug-induced Hepatotoxicity of Antitubercular Agents in Rats. Pharmacognosy magazine. 2018 Apr-Jun;14(54):180-5.
20. Altinoz E, Oner Z, Elbe H, Uremis N, Uremis M. Linalool exhibits therapeutic and protective effects in a rat model of doxorubicin-induced kidney injury by modulating oxidative stress. Drug Chem Toxicol. 2022 Sep;45(5):2024-2030. doi: 10.1080/01480545.2021.1894751.
21. Karlson AG, Ulrich JA. Stability of rifampin in dimethylsulfoxide. Applied microbiology. 1969 Oct;18(4):692-3.
22. Oh CT, Moon C, Choi TH, Kim BS, Jang J. Mycobacterium marinum infection in Drosophila melanogaster for antimycobacterial activity assessment. The Journal of antimicrobial chemotherapy. 2013 Mar;68(3):601-9.
23. Langnaese K, John R, Schweizer H, Ebmeyer U, Keilhoff G. Selection of reference genes for quantitative real-time PCR in a rat asphyxial cardiac arrest model. BMC molecular biology. 2008 May 28;9:53.
24. Aydın G, Adaleti R, Boz ES, Yücel FM, Özhan HK, Aksaray S. Investigation of Anti-HCV S/CO Value in Detecting Viremia in Patients with Hepatitis C Virus Infection. Mikrobiyoloji bulteni. 2020 Jan;54(1):110-9.
25. Kitamura R, Matsuoka K, Nagayama S, Otagiri M. Time-dependent induction of rat hepatic CYP1A1 and CYP1A2 expression after single-dose administration of the anti-angiogenic agent TSU-68. Drug metabolism and pharmacokinetics. 2008;23(6):421-7.
26. Refaiy A, Muhammad E, ElGanainy EO. Semiquantitative smoothelin expression in detection of muscle invasion in transurethral resection and cystectomy specimens in cases of urinary bladder carcinoma. African Journal of Urology. 2011 2011/03/01;17(1):6-10.
27. Gibson-Corley KN, Olivier AK, Meyerholz DK. Principles for valid histopathologic scoring in research. Veterinary pathology. 2013 Nov;50(6):1007-15.
28. Strobel H, Thompson C, Antonovic L. Cytochromes P450 in brain: function and significance. Current drug metabolism. 2001;2(2):199-214.

29. Morse DC, Stein AP, Thomas PE, Lowndes HE. Distribution and induction of cytochrome P450 1A1 and 1A2 in rat brain. Toxicology and applied pharmacology. 1998 Sep;152(1):232-9.
30. Singh AK, Kashyap MP, Jahan S, Kumar V, Tripathi VK, Siddiqui MA, et al. Expression and inducibility of cytochrome P450s (CYP1A1, 2B6, 2E1, 3A4) in human cord blood CD34(+) stem cell-derived differentiating neuronal cells. Toxicological sciences. 2012 Oct;129(2):392-410.
31. Maciejczyk M, Żebrowska E, Chabowski A. Insulin Resistance and Oxidative Stress in the Brain: What's New? Int J Mol Sci. 2019 Feb 18;20(4):874. doi: 10.3390/ijms20040874.
32. Li X, He X, Chen S, Le Y, Bryant MS, Guo L, et al. The genotoxicity potential of luteolin is enhanced by CYP1A1 and CYP1A2 in human lymphoblastoid TK6 cells. Toxicology letters. 2021 Jun 15;344:58-68.
33. Pyo MC, Shin HS, Jeon GY, Lee KW. Synergistic Interaction of Ochratoxin A and Acrylamide Toxins in Human Kidney and Liver Cells. Biological & pharmaceutical bulletin. 2020;43(9):1346-55.
34. Shimada T. Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. Drug metabolism and pharmacokinetics. 2006;21(4):257-76.
35. Tsuji G, Takahara M, Uchi H, Takeuchi S, Mitoma C, Moroi Y, et al. An environmental contaminant, benzo (a) pyrene, induces oxidative stress-mediated interleukin-8 production in human keratinocytes via the aryl hydrocarbon receptor signaling pathway. Journal of dermatological science. 2011;62(1):42-9.
36. Bartsch H, Rojas M, Alexandrov K, Camus AM, Castegnaro M, Malaveille C, et al. Metabolic polymorphism affecting DNA binding and excretion of carcinogens in humans. Pharmacogenetics. 1995;5:84-90.
37. Awodele O, Akintonwa A, Osunkalu VO, Coker HA. Modulatory activity of antioxidants against the toxicity of Rifampicin in vivo. Revista do Instituto de Medicina Tropical de Sao Paulo. 2010 Jan-Feb;52(1):43-6.
38. Malaplate-Armand C, Leininger-Muller B, Batt AM. Astrocytic cytochromes p450: an enzyme subfamily critical for brain metabolism and neuroprotection. Revue neurologique. 2004 Jul;160(6-7):651-8.
39. Khalil HE, Ibrahim HM, Darrag HM, Matsunami K. Insight into Analysis of Essential Oil from Anisosciadium lanatum Boiss.-Chemical Composition, Molecular Docking, and Mitigation of Hepg2 Cancer Cells through Apoptotic Markers. Plants. 2021 Dec 26;11(1):66. doi: 10.3390/plants11010066.
40. Caputo L, Piccialli I, Ciccone R, de Caprariis P, Massa A, De Feo V, Pannaccione A. Lavender and coriander essential oils and their main component linalool exert a protective effect against amyloid-β neurotoxicity. Phytother Res. 2021 Jan;35(1):486-493. doi: 10.1002/ptr.6827.
41. Jeong K, Shin YC, Park S, Park JS, Kim N, Um JY, et al. Ethanol extract of Scutellaria baicalensis Georgi prevents oxidative damage and neuroinflammation and memorial impairments in artificial senescense mice. Journal of biomedical science. 2011 Feb 8;18(1):14.
42. Gómez-Apo E, Silva-Pereyra J, Soto-Abraham V, Mondragón-Maya A, Sanchez-Lopez J. Immunohistochemical analysis of caspase expression in the brains of individuals with obesity or overweight. Obesity science & practice. 2023 Apr;9(2):137-44.
43. Yeh SH, Huang WS, Chiu CH, Chen CL, Chen HT, Chi DY, et al. Automated Synthesis and Initial Evaluation of (4'-Amino-5',8'-difluoro-1'H-spiro[piperidine-4,2'-quinazolin]-1-yl)(4-[(18)F]fluorophenyl)methanone for PET/MR Imaging of Inducible Nitric Oxide Synthase. Molecular imaging. 2021;2021:9996125.
44. Çelik R, Mert H, Comba B, Mert N. Effects of cinnamaldehyde on glucose-6-phosphate dehydrogenase activity, some biochemical and hematological parameters in diabetic rats. Biomarkers. 2022 May;27(3):270-7.
45. Scheen AJ. Central nervous system: a conductor orchestrating metabolic regulations harmed by both hyperglycaemia and hypoglycaemia. Diabetes & metabolism. 2010 Oct;36 Suppl 3:S31-8.
46. Rysä J, Buler M, Savolainen MJ, Ruskoaho H, Hakkola J, Hukkanen J. Pregnane X receptor agonists impair postprandial glucose tolerance. Clinical pharmacology and therapeutics. 2013 Jun;93(6):556-63.
47. Perry W. Rifampicin, halothane and glucose as mediators of lysosomal enzyme release and tissue damage. Medical hypotheses. 1988 Jun;26(2):131-4.
48. Deepa B, Venkatraman Anuradha C. Effects of linalool on inflammation, matrix accumulation and podocyte loss in kidney of streptozotocin-induced diabetic rats. Toxicology mechanisms and methods. 2013 May;23(4):223-34.
49. Yang CM, Lin CC, Hsieh HL. High-Glucose-Derived Oxidative Stress-Dependent Heme Oxygenase-1 Expression from Astrocytes Contributes to the Neuronal Apoptosis. Molecular neurobiology. 2017 Jan;54(1):470-83.
50. Niemi M, Kivistö KT, Backman JT, Neuvonen PJ. Effect of rifampicin on the pharmacokinetics and pharmacodynamics of glimepiride. British journal of clinical pharmacology. 2000 Dec;50(6):591-5.