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Nebivolol Mitigate the Hepatic Expression Level of Inducible and Endothelial Nitric Oxide Synthase in Tamoxifen-Induced Oxido-Inflammatory Changes in Female Rats

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Abstract

Liver injury can arise post-exposure to drugs or their metabolites, herbal and dietary supplements. Tamoxifen is a famous drug used in breast cancer treatment. Long-term tamoxifen treatment has been associated with the development of hepatotoxicity. Oxidative stress, fatty changes, and inflammation are the major implicated mechanisms contributing to tamoxifen hepatotoxicity including the inducible nitric oxide synthase (iNOS)-mediated inflammatory pathway in addition to standard hepatotoxicity biomarkers like alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Nebivolol is a third-generation selective beta1-adrenergic receptor blocker with vasodilator characteristics and significant antioxidant activity. The present study was designed to investigate the possible protective role of nebivolol against rat hepatotoxicity induced by tamoxifen. Rats utilized in this study were randomized into 5 groups (6 rats per group); Group 1- (Control) rats received distilled water (5mL/kg b.w. orally) for 14 consecutive days. Group 2- Rats received tamoxifen (75mg/kg orally) on days 13 and 14 only. Group 3- Rats received Nebivolol (5 mg/kg orally for 14 consecutive days) and tamoxifen (75mg/kg orally) on days 13 and 14 only. Group 4- Rats received Nebivolol (8 mg/kg orally for 14 consecutive days) and tamoxifen (75mg/kg orally) on days 13 and 14 only. Group 5- Rats received Nebivolol (10mg/kg orally for 14 consecutive days) and tamoxifen (75mg/kg orally) on days 13 and 14 only. pre-administration of nebivolol at different doses with tamoxifen showed significant downregulation ($P<0.05$) in hepatic AST, ALT, and iNOS with overexpression of eNOS compared to corresponding levels in the tamoxifen-only treated group. In conclusion, this study demonstrated that pre-administration of nebivolol in different doses concomitantly with tamoxifen resulted in attenuation of its hepatotoxicity.

Keywords: Hepatotoxicity, Tamoxifen, Nebivolol, iNOS, eNOS.

Introduction

Drug-induced liver injury (DILI) refers to the occurrence of hepatic damage caused by the use of drugs, herbs, or xenobiotics [(1)]. Tamoxifen, which or TAM, is widely regarded as the standard adjuvant therapy for both early and late breast cancer Since its approval by the Food and Drug Administration in 1977 [(2)]. TAM's proposed mechanism of action involves the suppression of estradiol binding to the ligand-binding domain of the estrogen receptor alpha (ERα), resulting in conformational alterations that impede the interaction between the estrogen receptor and co-activator proteins [(3)].

Nevertheless, clinicians express concerns regarding the hepatotoxicity associated with Tamoxifen therapy. The metabolic activation of Tamoxifen occurs in the liver through the influence of cytochrome P450 enzymes, producing 4-hydroxytamoxifen and endoxifen metabolites. These metabolites are accountable for the anticancer properties exhibited by tamoxifen [(4)]. Research has demonstrated that Tamoxifen can stimulate the excessive generation of reactive oxygen species (ROS) during its metabolic processes. This phenomenon has been observed to result in adverse impacts on both cellular and mitochondrial membranes, as well as the release of apoptotic components into the cytoplasm and subsequent activation of caspase, ultimately leading to apoptosis, considering iNOS and cytokines contribute to the underlying mechanism of TAM-induced hepatotoxicity in female rats [(5)].

Nitric oxide (NO) and its byproducts significantly influence the liver's physiology and pathology. In a general context, it is widely observed that nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS) has a protective role in disease development. Conversely, NO generated by inducible nitric oxide synthase (iNOS) was

harmful .The liver sinusoidal endothelial cells (LSECs) play a crucial role in regulating intrahepatic sinusoidal vascular tone and blood flow, as well as exerting an anti-inflammatory impact through the continuous production of small quantities of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS) [6]. The presence of NO-derived eNOS has been found to inhibit the activation of hepatic stellate cells (HSCs) and Kupffer cells, hence maintaining their quiescent state [7]. In pathological states, endothelial nitric oxide synthase (eNOS) activity tends to decrease, the reduction in NO-derived eNOS in liver sinusoidal endothelial cells (LSECs) leads to the capillarization of endothelial cells and the activation of hepatic stellate cells (HSCs) accompanied by the deposition of extracellular matrix (ECM), and the activation of kupffer cells leading to the elevation of inducible nitric oxide synthase (iNOS) expression and the exacerbation of the inflammatory event [6].

The adverse effects associated with these treatment adherence measures (TAM) can negatively impact patient adherence, resulting in the termination of treatment and subsequently leading to inferior clinical outcomes [8]. Therefore, it's crucial to investigate novel approaches to enhance TAM's safety profile, particularly for patients with chronic conditions such as hypertension who require beta blockers.

Nebivolol is classified as a third-generation β-blocker due to its vasodilator properties. The substance under consideration is a racemic combination of two enantiomers, l-nebivolol and d-nebivolol, in a 1:1 ratio. Nebivolol confers health benefits through the reduction of oxidative stress, achieved by two mechanisms: non-receptor-dependent scavenging of the superoxide radical [9] and β 3 -adrenergic receptor-dependent inhibition of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) [10]. Furthermore, it has been observed that Nebivolol enhances the bioavailability of nitric oxide (NO) in the vasculature through the activation of endothelial nitric oxide synthase (eNOS) [9].

The primary objective of the present study is to examine the impact of B-blocker (Nebivolol) on tamoxifen-induced hepatotoxicity in rats, specifically focusing on its effect on i/e NOS then antioxidant and anti-inflammatory propertie

Materials and Methods

Chemicals

Nebivolol (NEB) and tamoxifen (TAM) were purchased from Sigma-Alorich USA. The polyclonal rabbit/antirat iNOS, polyclonal rabbit/anti-rat eNOS anti-bodies, and Step Plus Poly-HRP Anti Mouse/Rabbit IgG detection system were from Elabsciense. Alanine transaminase (ALT) and aspartate transaminase (AST) were from Sigma-Aldrich USA.

Animals

This study utilized a sample of 30 healthy adult female albino rats, with weights ranging from 150 to 240 grams, and ages between 8 and 12 weeks. The experiment was conducted at the Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR) affiliated with Mustansiriyah University. The animals were subjected to controlled environmental circumstances, including a temperature range of 24 ± 2 degrees Celsius and a light cycle of 12 hours of light followed by 12 hours of darkness. During this period, the animals were provided unrestricted access to pellets and water ad libitum.

Experimental design

Animals were formatted into five equal groups, with six rats assigned to each group.

Group 1 (control): The rats received an oral administration of distilled water (DW) (5mL/kg) for 14 days.

In Group 2, the rats were administered distilled water (DW) for 12 days, followed by the oral administration of tamoxifen (TAM) at a dosage of 75mg/kg body weight on days 13 and 14, as previously described [11].

In Group 3 (NEB5), the rats were orally administered nebivolol at a dosage of 5 mg/kg body weight [12] for 14 days. Additionally, tamoxifen was administered orally at 75 mg/kg body weight on days 13 and 14 only.

In Group 4 (NEB8), the rats were administered nebivolol orally at an 8mg/kg body weight [13] dose for 14 days. Additionally, tamoxifen was administered orally at 75mg/kg body weight on days 13 and 14 only.

In Group 5 (NEB10), rats were orally administered nebivolol at 10 mg/kg body weight [14] for 14 days. Additionally, tamoxifen was administered orally at 75 mg/kg body weight on days 13 and 14 only.

Sample collection and preparation

The rats were sedated at the end of the experiment by administering 50 mg/kg ketamine and 5 mg/kg xylazine intramuscularly. A 5 mL syringe was used to draw blood samples from the heart. For serum separation, samples were put in gel tubes and centrifuged at 2500 rpm for 15 minutes at room temperature. The blood was collected in an Eppendorf tube and kept at (-20 °C) for estimating hepatic function parameters. The liver tissues were promptly dissected out, cleansed of adherent tissues, and rinsed with distilled water. Parts of each group's livers were stored in 10% formalin for immunohistochemical tests.

Liver function analysis

The assessment of liver function involved the analysis of serum samples for levels of alanine

aminotransferase (ALT) and aspartate aminotransferase (AST). This was carried out using colorimetric kits obtained from Sigma-Aldrich, in accordance with the instructions supplied by the manufacturers [15,16].

Immunohistochemistry

A portion of the liver was extracted and afterward preserved in a 10% formalin solution. The preserved liver sample was then embedded in paraffin. Sections with a thickness of five micrometers were carefully positioned onto slides that had a positive charge. The sections underwent deparaffinization using xylene, followed by rehydration using graded ethyl alcohol. The endogenous peroxidase activity was rendered inactive through a 30-minute treatment with a 3% hydrogen peroxide solution, followed by a subsequent washing step using a phosphate-buffered saline (PBS) solution. To facilitate antigen retrieval, the sections were subjected to a 20-minute boiling process in a microwave using citrate buffer with a pH of 6.0. The sections were subjected to overnight incubation in a humidity room with primary antibodies targeting iNOS and eNOS. Following this, a biotinylated secondary antibody was applied for a duration of 30 minutes. The sections were subjected to a wash using phosphate-buffered saline (PBS) and afterward exposed to the streptavidin-biotin complex reagent for a duration of 30 minutes. Subsequently, a solution of 3,3-diaminobenzidine tetrahydrochloride (DAB) was administered for a duration of 5 minutes. Following this, the sample was rinsed with distilled water, subjected to counterstaining using Mayer's hematoxylin, dried, treated with xylene for clarification, and ultimately mounted and covered with a cover [17].

The sections were subjected to screening using a light microscope at a magnification of 200X. To evaluate the presence of positive staining for endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS), the sections were examined for cytoplasmic staining using the following score system [18] illustrated in Table 1.

Statistical Analysis

The data underwent analysis utilizing SAS (Statistical Analysis System - version 9.1). The present study utilized a one-way analysis of variance (ANOVA) as the statistical method, followed by the application of the Least Significant Differences (LSD) test for post hoc analysis. The present study employed this methodology to assess and ascertain any notable differences among the means of the variables under scrutiny. Post hoc tests are an essential component of the analysis of variance (ANOVA) methodology. The statistical significance of the data differences was assessed at a significance level of $P<0.05$ [19].

Table 1. Immunohistochemical staining score system.

Grade	Percent of cells positive approach	Stain intensity
0	No staining	Negative
1	1-25% reactive cells stained positive.	Weak
2	26-50% of cells stained positive.	Moderate
3	51-75 % of cells stained positive.	Strong
4	76-100% cells stained positive.	Intense

Results and Discussion

Assessment of hepatic enzymes

The rats that received tamoxifen treatment in the induction group exhibited a statistically significant elevation (P-value < 0.05) in serum AST and ALT concentrations compared to the control group. The study found that the hepatic enzyme levels of AST and ALT were significantly decreased (P-value < 0.05) in the NEB5, NEB8, and NEB10 groups that underwent pretreatment with nebivolol, compared to the induction group. The evidence is reported in Table 2 Figures 1 and 2.

Table 2. Hepatic enzyme changes in all groups.

Groups	AST (IU/L)	ALT (IU/L)
G1:Control	135.33±9.85d	91.33±9.61c
G2;Induction	350.33±23.98a	164.00±9.28a
G3:NEB/5mg	265.83±5.54b	118.83±6.84b
G4:NEB/8mg	230.00±9.77b	116.66±4.93b
G5;NEB/10mg	186.66±11.10c	100.66±4.85bc
LSD	39.55	21.53

The results were expressed as Mean ±SD. Results with unidentical superscripts (a, b, c, d) are significantly different ($p<0.05$). LSD: a least significant difference.

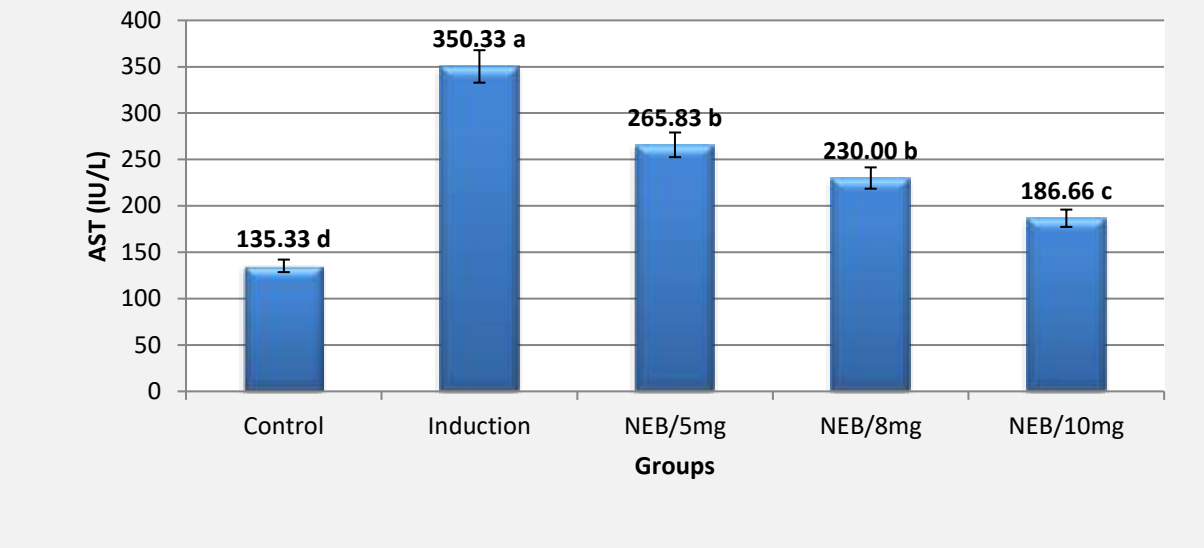

Figure 1. Bar chart showing levels of AST (IU/L) in different experimental groups. Control: normal control group, rats given distilled water for 14 days; Induction: negative control group, rats exposed to Tamoxifen on days13 and 14 only; NEB/5mg: Rats treated with nebivolol (5mg/kg/d) orally plus tamoxifen on days13 and 14 only ; Neb/8mg : Rats treated with nebivolol (8mg/kg/d) orally plus tamoxifen on days13 and 14 only; NEB/10mg: Rats treated with nebivolol (10mg/kg/d) orally plus tamoxifen on days13 and 14 only.

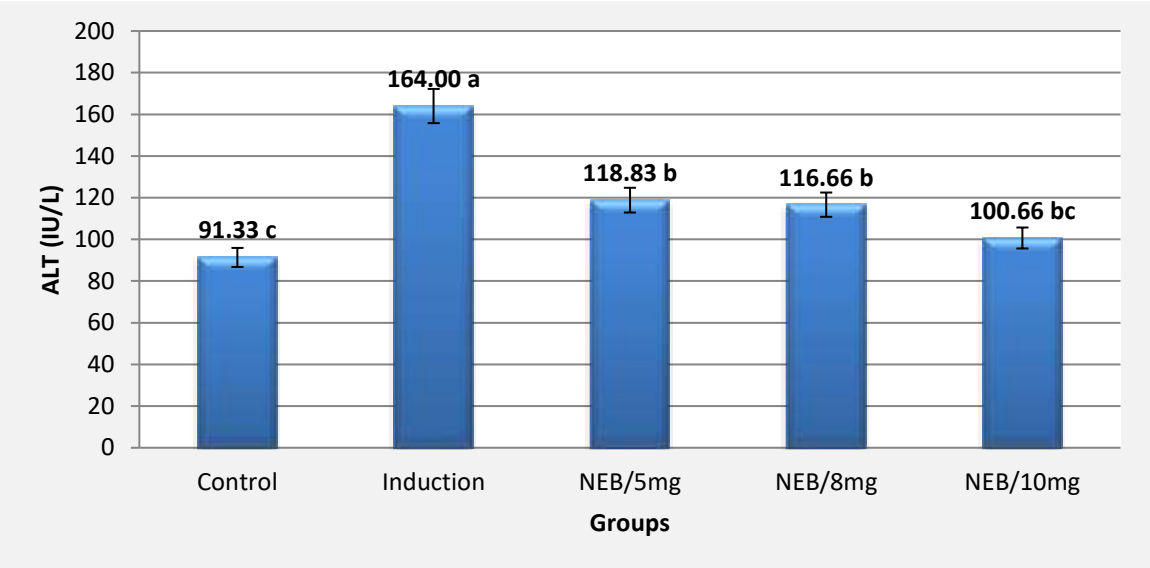

Figure 2. Bar chart showing levels of AST (IU/L) in different experimental groups. Control: normal control group, rats given distilled water for 14 days; Induction: negative control group, rats exposed to Tamoxifen on days13 and 14 only; NEB/5mg: Rats treated with nebivolol (5mg/kg/d) orally plus tamoxifen on days13 and 14 only; Neb/8mg: Rats treated with nebivolol (8mg/kg/d) orally plus tamoxifen on days13 and 14 only; NEB/10mg: Rats treated with nebivolol (10mg/kg/d) orally plus tamoxifen on days13 and 14 only

Immunohistochemical expression of eNOS and iNOS.

Regarding the immunoreactivity of eNOS, the results demonstrate a statistically non-significant decrease in eNOS immunohistochemistry expression(P-value > 0.05) in the group exposed to TAM compared to the control group. The NEB+ TAM groups exhibited a statistically significant increase in (P-value < 0.05) its expression compared to the group that received only TAM in a dose-dependent manner as shown in Table3 Figure3and 5.

Regarding iNOS immunoreactivity: The results indicate a statistically significant rise(P-value < 0.05) in iNOS immunohistochemistry expression in the group treated with TAM compared to the control group. The NEB+ TAM group exhibited a statistically significant decrease (P-value < 0.05) in expression compared to the group that received TAM alone as obtained in Table 3, Figure4 and 6.

Table 3. eNOS, iNOS expression levels in all groups.

Groups	eNOS Grade (% of immunopositive cell/field)	iNOS Grade (% of immunopositive cell/field)
G1:Control	1.00±0.00ab	0.00±0.00c
G2:Induction	0.00±0.00b	4.00±1.00a
G3:NEB/5mg	2.00±1.00ab	3.00±1.00ab
G4:NEB/8mg	3.00±1.00a	2.00±0.00abc
G5:NEB/10mg	3.00±0.00a	1.00±0.00bc
LSD	2.29	2.30

The results were expressed as Mean ±SD. Results with unidentical superscripts (a, b, c, d) are significantly different ($p<0.05$). LSD: a least significant difference.

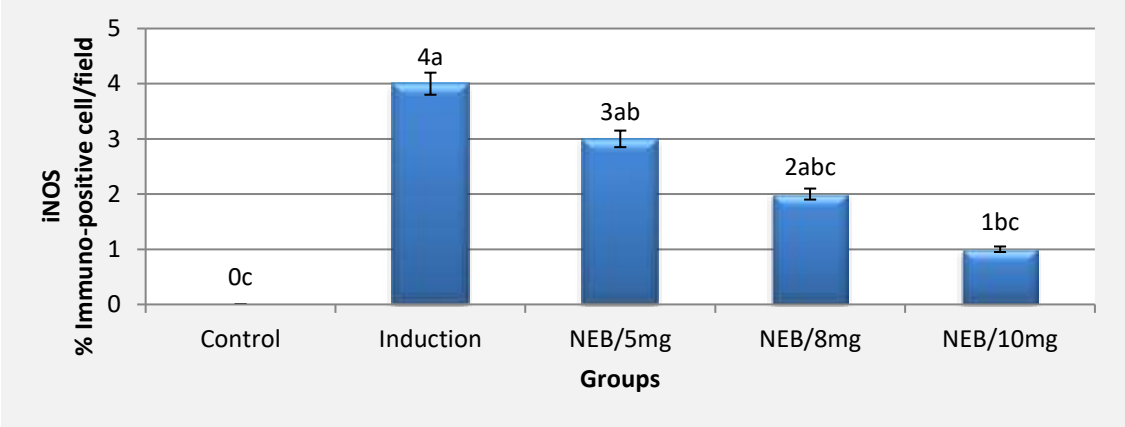

Figure 3. Bar chart showing the Semiquantitative analysis of Nebivolol on inducible nitric oxide synthase immunoexpression changes in tamoxifen-induced hepatotoxicity. Control: normal control group, rats given distilled water for 14 days; Induction: negative control group, rats exposed to Tamoxifen on days13 and 14 only; NEB/5mg: Rats treated with nebivolol (5mg/kg/d) orally plus tamoxifen on days13 and 14 only ; Neb/8mg : Rats treated with nebivolol (8mg/kg/d) orally plus tamoxifen on days13 and 14 only; NEB/10mg: Rats treated with nebivolol (10mg/kg/d) orally plus tamoxifen on days13 and 14 only.

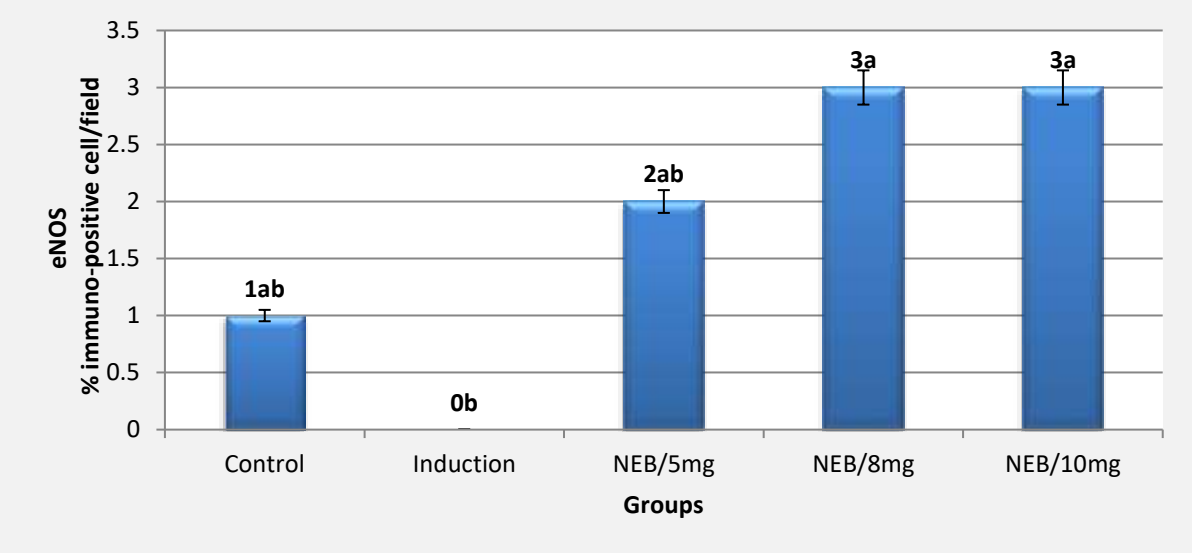

Figure 4. Bar chart showing the Semiquantitative analysis of Nebivolol on endothelial nitric oxide synthase immunoexpression changes in tamoxifen-induced hepatotoxicity. Control: normal control group, rats given distilled water for 14 days; Induction: negative control group, rats exposed to Tamoxifen on days13 and 14 only; NEB/5mg: Rats treated with nebivolol (5mg/kg/d) orally plus tamoxifen on days13 and 14 only ; Neb/8mg : Rats treated with nebivolol (8mg/kg/d) orally plus tamoxifen on days13 and 14 only; NEB/10mg: Rats treated with nebivolol (10mg/kg/d) orally plus tamoxifen on days13 and 14 only.

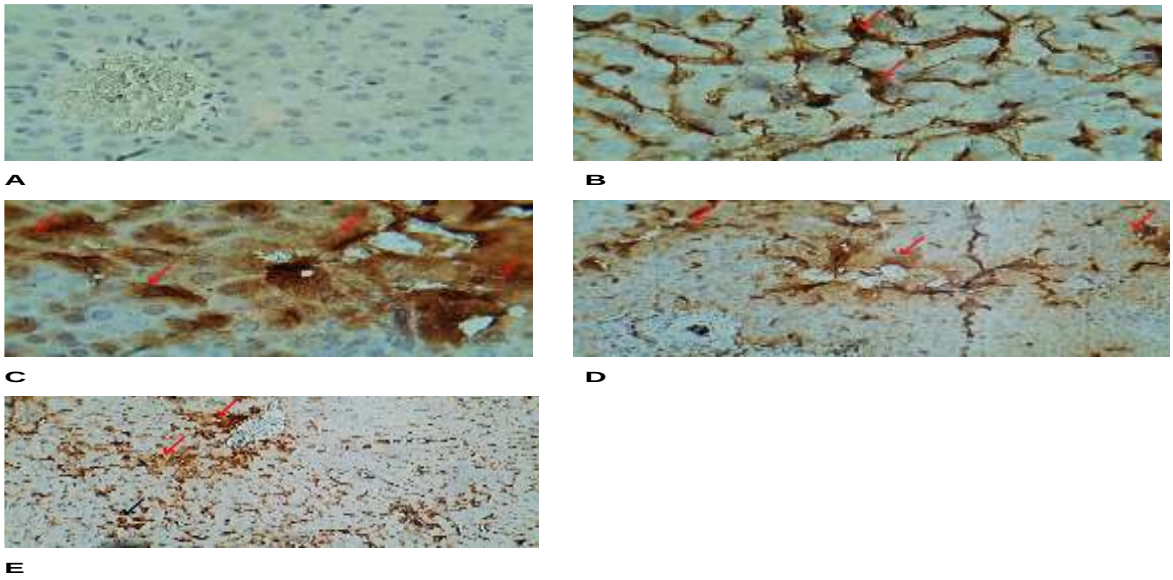

Figure 5: Effect of Nebivolol on inducible nitric oxide synthase immunoexpression in tamoxifen-induced hepatotoxicity. A: Control:rats given distilled water for 14 days = grade 0 (no positive reaction) B: Induction: rats exposed to Tamoxifen on days13 and 14 only =grade 4 (positive reaction within endothelial of sinusoid and kupffer cells /intense intensity(Arrows)) C: NEB/5mg: Rats treated with nebivolol (5mg/kg/d) orally plus tamoxifen on days13 and 14 only = grade 3 (positive reaction at central zone of hepatocyte with strong intensity(Arrows)), D: NEB/8mg: Rats treated with nebivolol (8mg/kg/d) orally plus tamoxifen on days13 and 14 only = grade 2 (Moderate positive reaction within hepatocytes at level of central zone (Arrows) E: NEB/10mg: Rats treated with nebivolol (10mg/kg/d) orally plus tamoxifen on days13 and 14 only = grade 1 (little of positive reaction at central zone (red arrows) and peripheral zone of hepatic cords (Black arrow) with weak intensity)

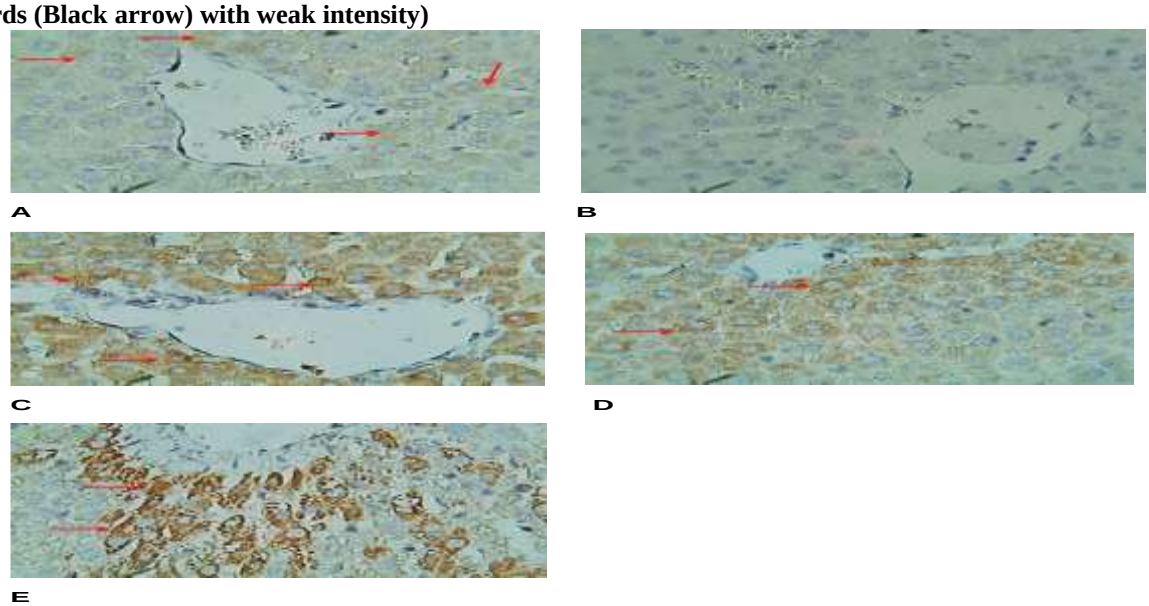

Figure 6. Effect of Nebivolol on endothelial nitric oxide synthase immunoexpression in tamoxifen-induced hepatotoxicity. A: Control: rats given distilled water for 14 days = grade 1 (little spots of weak positive reaction within hepatocytes at level of central zone (Arrows), B: Induction: rats exposed to Tamoxifen on days13 and 14 only = grade 0 (no positive reaction), C: NEB/5mg: Rats treated with nebivolol (5mg/kg/d) orally plus tamoxifen on days13 and 14 only = Grade 2 (a an area extended from central to peripheral zone of hepatocytes revealed positive reaction and moderate intensity (Arrows), D: NEB/8mg; Rats treated with nebivolol (8mg/kg/d) orally plus tamoxifen on days13 and 14 only = Grade 3 (a wide area extended from central to peripheral zone of hepatocytes revealed positive reaction and sever intensity (Arrows), E: NEB/10m: Rats treated with nebivolol (10mg/kg/d) orally plus tamoxifen on days13 and 14 only = Grade 3 (a an area extended from central to peripheral zone of hepatocytes revealed positive reaction and sever intensity (Arrows).

Discussion

Liver injury can occur with exposure to medications or their metabolites, as well as herbal and nutritional supplements [1]. Previous research has primarily concentrated on investigating the potential preventive properties of antioxidant and anti-inflammatory substances against hepatotoxicity [20,21].

Tamoxifen has been widely utilized as the preferred pharmaceutical intervention for treating and preventing breast cancer, with an average prescription duration ranging from 3 to 5 years. Unfortunately, epidemiological studies have indicated that 40% of patients acquired hepatotoxicity through steatohepatitis after two years [22]. Additionally, in Iraq, a case-control study conducted at a hospital revealed that 86% of patients treated with tamoxifen experienced fatty liver [23].

Nebivolol is a pharmacological agent used to treat hypertension, with notable antioxidant and anti-inflammatory properties[9]. Also, recent studies have revealed the tumour-inhibiting effects of nebivolol in several types of malignancies, including breast cancer [24].

This investigation aimed to assess the potential hepatoprotective properties of nebivolol in mitigating Tamoxifen-induced steatohepatitis and ameliorating the biochemical and morphometrical aberrations observed in the liver of female albino rats.

In the current investigation, the oral administration of tamoxifen at a dosage of 75mg/kg led to notable increases in serum liver function enzymes, specifically alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in comparison to the healthy control group align with prior research cited as references [25] and [26]. The observed increase in elevation can potentially be attributed to structural damage in the liver, as these enzymes are typically found within the cytoplasm of hepatocytes and are released into the bloodstream following cellular damage. This could potentially be a result of the cytotoxic effects of TAM [27].

Pre-treatment with nebivolol results in a notable reduction in AST and ALT levels when compared to the induction group. This finding is consistent with prior researchs [28,29]. The observed phenomenon may be attributed to the anti-oxidant capabilities of nebivolol, which effectively inhibits the tamoxifen-induced oxidative damage of hepatocytes. Consequently, the leakage of hepatocellular contents such as ALT and AST into the circulation is prevented.

Tamoxifen-induced inflammation, apoptosis, and oxidative stress is mediated by inducible nitric oxide synthase (iNOS), a mediator of inflammation, this enzyme is produced by activated kuppfer cells in the liver and results in the overproduction of a high amount of NO and inflammation [5,30]. These studies are in agreement with our study that showed a significant increase in iNOS immunoexpression in the TAM induction group.

The present study showed that NEB pretreatment alleveate iNOS as seen in weak iNOS immunoexpression in NEB5, NEB8, and NEB10 groups, compared with high reactivity detected in TAM induction group in hand with previous studies [12,14]. This may be attributed to the nebivolol's ability to scavenge oxygen free radicals[9], an important promoter of inflammation by upregulation of nuclear factor-kappa B (NF-κB) and its down regulatory cytokines like tumor necrosis factor-alpha (TNFα) which activate iNOS, TNFα-NFκB and iNOS pathway involve in the pathogenesis of hepatotoxicity by tamoxifen[5]. another explanation is the nebivolol's ability to express and activate eNOS, this enzyme protects from liver disease by calming kuppfer cells the main source of iNOS enzyme in the liver [6] so thus cutting the road to iNOS activation. eNOS was expressed constitutively in sinusoidal and vascular endothelial cells to protect the liver this was seen in the control liver in the current study.

The Tamoxifen induction group shows non-significant eNOS downregulation due to the overproduction of ROS[5], oxidative stress may cause uncoupling of the endothelial nitric oxide synthase (eNOS), thus diminishing NO synthesis/bioavailability. Furthermore, the large amount of O2•−can reacts with NO to form peroxynitrite (ONOO−) which is a highly reactive and potent free radical that can decrease eNOS protein expression [6].

On the contrary, nebivolol addition to the treatment regimen in the third, fourth, and fifth groups of this study markedly increased eNOS immunoexpression following previous studies [12,14] which explained this protective effect of NEB in the liver that NEB is metabolized in the liver and its metabolites increase NO production in vascular endothelium leading to vasodilatation. NO increases hepatic microvascular blood flow by inducing smooth muscle relaxation in the terminal arterioles and portal vein. This creates an antiapoptotic effect in the liver [31] in addition to its inflammatory effect by keeping kuppfer cells inactive and HSs quiescent [7].

According to our study; NEB administration improved TAM-induced hepatotoxicity due to different mechanisms. In addition to the previously discussed mechanisms; NEB could significantly decrease the increased blood pressure and preserve vascular endothelium to maintain a normal blood supply, maintain the required oxygen supply to organs, and protect blood vessels from the harmful effect of increasing blood pressure including vessel damage, increasing oxidative stress, apoptosis and inflammation. Systemic increase in blood pressure

is associated with portal hypertension that leads to liver damage [31,32].

Conclusion

In this work, we aimed to assess the potential protective effect of NEB (Nebivolol) in hepatotoxicity produced by TAM (tamoxifen). This investigation represents an initial step toward further evaluating the impact of NEB on human subjects. The administration of NEB showed protective effects against tamoxifen-induced hepatotoxicity in rats, likely attributed to its anti-oxidant anti-inflammatory action mainly by eNOS activation and iNOS inhibition. Consequently, in addition to the previously documented additive anticancer effects of nebivolol, combination therapy of NEB and TAM may offer complementary benefits regarding hepatic safety. As such, this combination needs to be further studied clinically to treat hypertensive patients who are at risk for liver damage, such as those with breast cancer who are taking tamoxifen as a regimen.

Acknowledgment

Mustansiriyah University has been instrumental in our ability to complete this project, and we'd like to take this chance to express our gratitude.

Conflicts of Interest

There is no conflict of interest regarding the publication of this work.

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Ethics Statements

The study was approved by the ethics committee of College of Pharmacy/ Mustansiriyah University (acceptance number 8 on 11/10/2023).

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design:Yassir Mustafa kamal and huda jabber waheed,; data collection: noor ahmed hammadi; analysis and interpretation of results: yassir Mustafa kamal, huda jabber waheed, noor ahmed hammadi ; draft manuscript preparation: huda jabber waheed, noor ahmed hummadi.. All authors reviewed the results and approved the final version of the manuscript.

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يعدل نيبيفولول من مستويات التعبير الكبدي لمخلقة اوكسيد النتريك المحفز والتاسيسي ويخفف من التغيرات المؤكسدة الالتهابية الناجمة عن عقار تاموكسيفين في إناث الجرذان

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الخلاصة

يمكن أن تنشأ إصابة الكبد بعد التعرض للأدوية أو مستقلباتها والمكملات العشبية والغذائية. عقار تاموكسيفين هو دواء مشهور يستخدم في علاج سرطان الثدي. ارتبط العلاج طويل الأمد بعقار تاموكسيفين بتطور السمية الكبدية .ويعتبر الإجهاد التأكسدي والتغيرات الدهنية والالتهاب من الآليات الرئيسية المتورطة التي تساهم في السمية الكبدية لعقار تاموكسيفين بما في ذلك مخلقة أكسيد النتريك المحفز بالاضافة الى المؤشرات الحيوية القياسية للتسمم الكبدي مثل ناقلةامين الالانين وناقلة امين الاسبارتات.تم تصميم الدراسة للتحقيق في الدور الوقائي المحتمل للنيبيفولول ضد السمية الكبدية للفئران التي يسببها عقار تاموكسيفين.حيث تم تقسيم الجرذان المستخدمة في هذه الدراسة بصورة عشوائية إلى ٥ مجموعات (٦ فئران لكل مجموعة). تلقت المجموعة الأولى (مجموعة المقارنة) فئران التجارب الماء المقطر (٥ مل / كجم وزن حي عن طريق الفم) لمدة ١٤ يومًا متتاليًا. في المجموعة الثانية: تلقت الجرذان عقار تاموكسيفين (٧٥ مجم / كجم وزن حي ، عن طريق الفم) في الأيام ١٣ ، ١٤ فقط.وفي المجموعة الثالثة: تلقت الجرذان النيبيفولول (٥ مجم / كجم من وزن الجسم ، عن طريق الفم لمدة ١٤ يومًا متتاليًا) وتاموكسيفين (٧٥ مجم / كجم من وزن الجسم ، عن طريق الفم) في الأيام ١٣ ، ١٤ فقط. وفي المجموعة الرابعة: تلقت الجرذان نيبفولول (٨ مجم / كجم من وزن الجسم ، عن طريق الفم لمدة ١٤ يومًا متتاليًا) وتاموكسيفين (٧٥ مجم / كجم من وزن الجسم ، عن طريق الفم) في الأيام ١٣ ، ١٤ فقط. وفي المجموعة الخامسة: تلقت الجرذان نيبفولول (١٠ مجم / كجم من وزن الجسم ، عن طريق الفم لمدة ١٤ يومًا متتاليًا) وتاموكسيفين (٧٥ مجم / كجم من وزن الجسم ، عن طريق الفم) في الأيام ١٣ ، ١٤ فقط. أظهر التناول المسبق للنيبيفولول بجرعات مختلفة مع عقار تاموكسيفين تنظيمًا هابطًا ملحوظًا في ناقلة أمين الألانين وناقلة أمين الأسبارتات بالاضافه الى مخلقة أكسيد النتريك المحفزمع تصاعدا ملحوظا لمخلقة أكسيد النتريك التاسيسي مقارنةً بالمستويات المقابلة في المجموعة المعالجة بالتاموكسيفين فقط. في الختام، أظهرت هذه الدراسة أن التناول المسبق للنيبيفولول بجرعات مختلفة مع عقار تاموكسيفين أدى إلى تخفيف سميته الكبدية.

الكلمات المفتاحية: السمية الكبدية، تاموكسيفين، نيبيفولول،eNOS، iNOS