The Ameliorate Effects of Nerolidol on Thioasteamide-Induced Oxidative Damage in Heart and Kidney Tissue

Kalp ve Böbrek Dokusunda Tiyoasteamid Kaynaklı Oksidatif Hasarın Üzerine Nerolidolün Koruyucu Etkileri

Abstract

INTRODUCTION: Objective(s): Thioacetamide (TAA) is an organosulfur, white crystalline compound having liver injury. However, it shows toxic effects on many organs. The reverts the oxidative stress created by TAA on the heart and kidney, and decreased lipid peroxide peroxidation back with antioxidant-properties nerolidol. This study hypothesized that NRL treatment a potential ameliorate nephrotoxicity and cardiotoxicity caused by TAA.

METHODS: Materials and Methods: 32 Wistar Albino male rats (3-4 months old and 280-30 g in weight) were divided into four groups. (a) Control, (b) TAA was administered 200 mg/kg ip twice a weekly (c) NRL was orally administered at the dose of 100 mg/kg per every other day by gavages. (d) TAA and NRL treated group were assigned 200 mg/kg TAA and 100 mg/kg NRL for three weeks.

RESULTS: Results: As a result of these dose administration thiobarbituric acid reagent (TBARS) levels, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GSH-Px) levels were detected. The results were shown that TAA leads to a significant rise in TBARS level and a significant decrease in GPX, CAT, SOD, and GSH levels in the heart and kidney tissue according to the control group. The finding of this study the nerolidol treatment reduce TBARS levels and increases antioxidant level.

DISCUSSION AND CONCLUSION: Conclusion: The findins of the present study show that the antioxidant activity of NRL can protect against biochemical and histological damage caused by TAA in heart and kidney tissue.

Keywords: Nerolidol, Thioacetamide, Lipid peroxidation, Nephrotoxicity, Cardiotoxicity

Öz

GİRİŞ ve AMAÇ: Amaç: Tiyoasetamid (TAA), karaciğere zarar veren beyaz kristalli bir organosülfür bileşigidir. Ancak birçok organ üzerine toksik etkiler gösterir. Antioksidan özelliklileri ile bilinen Nerolidol Tiyoasetamitin kalp ve böbrek dokularında neden olduğu oksidatif stresi önler ve lipid peroksidasyonunu azaltır. Bu çalışmanın amacı, Nerolidolün TAA kaynaklı nefrotoxisite ve kardiyotoksisitesinin ratların üzerindeki koruyucu etkisi araştırılmıştır.

YÖNTEM ve GEREÇLER: Gereç ve Yöntemler: 32 adet Wistar Albino erkek siçan (3-4 aylık ve ağırlıkları 280-30 gr) dört gruba ayrıldı. (a) Kontrol gruba, (b) TAA gruba haftada iki kez 200 mg / kg ip olarak uygulandı (c) NRL grubu, gavaj yoluyla her gün 100 mg / kg dozunda oral yoldan uygulandı. (d) TAA ve NRL grubu, üç hafta süreyle 200 mg / kg TAA ve 100 mg / kg NRL uygulandı.
Introduction

Nowadays, it has caused an increase in serious organ damage as a result of acute and chronic exposure to toxic chemicals. Thioacetamide (TAA), is a chemical used in industrial areas such as leather, textile, and paper and in laboratories as an organic solvent. TAA has significant toxic effects on organs such as the liver, kidney, spleen, lung, intestine, stomach, and brain, causing structural and functional modification. TAA is metabolized in vivo to free radical derivatives, TAA sulfoxide and TAA-S, S-dioxide, resulting in increased lipid peroxidation, resulting in ROS formation and thus multi-organ damage. Increased free radical development or reduced free radical scavenging is responsible for oxidative stress. Oxidative stress is a major imbalance between free radical development and defense mechanisms against antioxidants. Pro-oxidant agents are primarily constituted by reactive oxygenated (ROS) and nitrogenous species (RNS).

The generation of a large amount of ROS due to TAA can inhibit the antioxidant defense mechanism. TAA can damage cellular ingredients such as lipids, proteins, and DNA; this can impair cellular structure and function. Intracellular antioxidant system compounds (like GSH and other thiols) can insufficient to proper this damage. The damage of TAA on the heart was evaluated based on the oxidative stress both biochemically and histologically. The heart damage is characterized by ROS occurrence, lipid peroxidation, and adverse impacts on the antioxidant-oxidant system. Some studies have shown that oxidative stress plays an important role in TAA-induced toxicity. However, it has been suggested that various antioxidant treatments show beneficial effects by reducing oxidative stress.

Recently, in addition to modern treatment methods, plant-based treatments have become more important. The progression of technology and the serious side effects of pharmaceutical agents used in medical treatment have increased the interest in medicinal plants and enabled the investigation of bioactive compounds found in these plants. Natural components are a present source of antioxidants and many researchers based on discovering new antioxidant compounds from plants. An increasing number of studies have shown that essential oils obtained from medicinal plants exhibit a variety of biological properties. Antioxidants found in plants are being investigated to treat many disorders such as cardiovascular diseases, cancer, and neurological condition. Nerolidol (NRL), also known as ‘3,7,11-trimethyl-1,6,10-dodecatrien-3-ol’ is an aliphatic xylene alcohol derived from many plants with antioxidant properties. Nerolidol (NRL) is found in different plant species, Ferula fukanensis, Baccharis dracunculifolia, Amaranthus retroflexus and Canarium schweinfurthii. NRL, also known as 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol is an aliphatic xylene alcohol derived from many plants with antioxidant properties. The antioxidant, radical scavenging, and anti-inflammatory effects of nerolidol have been demonstrated in several studies. Nogueira et al. (2013) found that essential oils from Ferula fukanensis containing nerolidol induced a decrease in nitric oxide production and restricts gene expression NO-induced. In this context, nerolidol can be suggested utilization as an antioxidant agent. Although there is currently limited information about the bioactivity of essential oils, it easily crosses the cell membrane and can interact with intracellular proteins. Therefore, many studies have implicated the free radical scavenging properties of essential oils. Studies have shown the antioxidant, radical scavenging, and anti-inflammatory effects of NRL. Additionally, Javed et al. (2016) found that administration of nerolidol (30 mg/kg, i.p.) to rats reversed inflammation and oxidative stress by increasing levels of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and decreasing lipid peroxidation and thiobarbituric acid reactive substances (TBARS) levels. In this context, Nogueira-Neto et al. (2013) found that treatment with nerolidol (75 mg/kg, i.p.) decreased oxidative stress in the mouse, resulting in increased SOD and CAT activity. Previous studies have suggested that therapeutic potential of nerolidol to treat and prevent diseases associated with oxidative stress. According to the literature, there is no previous study that investigated the efficacy of NRL on cardiac ameliorate. Therefore, this study aimed to investigate the protection of NRL, which is thought to have high antioxidant potential against the cardiotoxic and nephrotoxic effects of TAA. For this purpose, histopathological and biochemical effects of TAA on the heart and kidney were examined and the protective effect of NRL on these parameters was investigated.
Material method

Animals and Treatment:
The present study was approved by the Ethics Committee on Animal Research of Pamukkale University (protocol number: 2020/20) and carried out in accordance with The Guidelines for Animal Research from the National Institutes of Health (NIH). Wistar Albino male rats weighing 280-300 g were supplied by the Pamukkale University Laboratory Animals Research Center (Denizli, Turkey), housed in sterilized polypropylene cages, and given an ad libitum diet of standard commercial food pellets and water. Animals were randomly divided into 4 groups with eight animals in each group:

- Group 1-control, was given isotonic saline (intraperitoneal) and corn oil administered oral gavage every other day;
- Group 2- Thioacetamide (TAA), 200 mg/kg, intraperitoneal (i.p.) TAA was given two times per week for three weeks, and 0.9% NaCl was administered i.p. as a single dose at the beginning;
- Group 3-NRL 100 mg/kg NRL (Merck, CAS No: 7212-44-4), was given by gavages every other day, and corn oil was given by gavages daily;
- Group 4-TAA + NRL, 200 mg/kg TAA was given i.p. two times per week, and 100 mg/kg NRL, suspended in corn oil, was given by gavages every other day. After three weeks of treatment, all rats were euthanized under anesthesia. Heart and kidney tissues samples were collected for biochemical analyses and histological examination.

Biochemical assay:

Tissues were homogenized for biochemical parameters examination. TBARS level tissues homogenates were determined using a spectrophotometric process that is focused on the reaction between thiobarbituric acid and 25. 26 GSH levels of tissues were determined at 412 nm according to the Sedlak and the Lindsay method.27 The results were expressed as nmol/mL. Superoxide dismutase (SOD) activity was determined by the method of Sun et al. using spectrophotometrically. SOD enzyme activity was determined in the inhibition of nitro blue tetrazolium (NBT) depend on xanthine/xanthine oxidase enzyme activity.28 The tissues were measured at 560 nm in spectrophotometer CAT level of tissues were determined by H2O2 consumption according to the Aebi method.29 Glutathione peroxides (GPx) level was determined as a spectrophotometrically using method of Paglia & Valentine.30 The tissue protein content was calculated by the method of Lowry et al.31

Histopathological Assay:

For light microscopic evaluation, liver and brain samples were fixed in 10 % formalin. The tissue samples were processed by routine tissue techniques and were embedded in paraffin. Paraffin-embedded specimens were cut into 5 mm thick sections, mounted on slides and stained with Hematoxylin- Eosin (H-E). Stained sections were examined under a Leica DFC280 light microscope by Leica Q Win and Image Analysis System. We examined heart sections for hemorrhage, necrosis, vascular congestion, vacuolisation, mononuclear cell infiltration, oedema and eosinophilic stained and pyknotic nuclei cells. We examined kidney sections for inflammatory cell infiltration, hemorrhage, glomerular degeneration, vascular congestion, hemorrhage between the tubules, vacuolization of tubular epithelial cells and oedema between the tubules, epithelial atrophy, and cell desquamation in the tubules and casts in tubular lumen. Histopathologic damage score was calculated these findings. Histopathologic damage score was calculated according to the degree of damage severity to 0 (none), 1 (mild), 2 (moderate), 3 (severe).

Statistical analysis:

ANOVA (post hoc Tukey test) was performed for comparing biochemical, histopathological, and immunohistochemical scores between the groups. Statistical calculations were carried out using the SPSS 18.0 program package (SPSS Inc., Chicago, IL, USA). Variables were presented as means ± standard deviations and p<0.05 was the gauge to indicate statistical significance. Histopathological data analysis were used SPSS 13.0 (SPSS Inc., Chicago, Ill., USA) and MedCalc 11.0 (Belgium) statistical programs. The data are expressed as arithmetic mean ± SE. Kruskal-Wallis and Conover tests were used. Exact p values were given where available, and p< 0.001 was considered as statistically significant.

Results:

Biochemical Results:

CAT, SOD, GPx, reduced GSH (antioxidant parameters), and TBARS (oxidant parameter) values in heart tissues are presented in Table 1. Mean heart tissue TBARS levels were 11.49±3.43 nmol/g tissue in the Control group. TAA administrated significantly increased TBARS levels to 17.42±1.64 nmol/g tissue in the TAA group. After NRL administration, TBARS levels significantly decreased to 8.44±0.28 nmol/g tissue in the TAA+ NRL group. Mean heart tissue GSH levels were 41.82±6.51 nmol/ml tissue in the Control group. TAA administrated significantly decreased GSH levels to 17.64±0.93 nmol/ml tissue in the TAA group. After NRL administration, GSH levels significantly increased to 27.58±1.04 nmol/ml tissue in the TAA+ NRL group. Mean heart tissue
CAT levels were 0.302±0.10 kU/mg protein tissue in the Control group. TAA administrated significantly decreased CAT levels to 0.004±0.0002 kU/mg protein tissue in the TAA group. After NRL administration, CAT levels increased to 0.009±0.003 kU/mg protein tissue in the TAA+ NRL group. Mean heart tissue SOD activities were 86.68±7.71 kU/mg protein tissue in the Control group. TAA administrated decreased SOD activities to 66.98±9.32 kU/mg protein tissue in the TAA group. After NRL administration, SOD activities increased to 78.96±13.18 kU/mg protein tissue in the TAA+ NRL group. Mean heart tissue GPx levels were 0.55±0.09 U/mg protein tissue in the Control group. TAA administrated significantly decreased GPx levels to 0.12±0.02 U/mg protein tissue in the TAA group. After NRL administration, GPx levels increased to 0.18±0.06 U/mg protein tissue in the TAA+ NRL group.

CAT, SOD, GPx, reduced GSH (antioxidant parameters), and TBARS (oxidant parameter) values in kidney tissues are presented in Table 2. Mean kidney tissue TBARS levels were 4.07±0.89 nmol/g tissue in the Control group. TAA administrated significantly increased TBARS levels to 9.45±0.76 nmol/g tissue in the TAA group. After NRL administration, TBARS levels decreased to 8.49±0.36 nmol/g tissue in the TAA+ NRL group. Mean kidney tissue GSH levels were 52.75±2.93 nmol/ml tissue in the Control group. TAA administrated significantly decreased GSH levels to 40.78±4.18 nmol/ml tissue in the TAA group. After NRL administration, GSH levels significantly increased to 59.48±3.79 nmol/ml tissue in the TAA+ NRL group. Mean kidney tissue CAT levels were 0.013±0.002 kU/mg protein tissue in the Control group. TAA administrated significantly decreased CAT levels to 0.007±0.001 kU/mg protein tissue in the TAA group. After NRL administration, CAT levels increased to 0.013±0.008 kU/mg protein tissue in the TAA+ NRL group. Mean kidney tissue SOD activities were 113.13±9.12 kU/mg protein tissue in the Control group. TAA administrated significantly decreased SOD activities to 42.79±16.16 kU/mg protein tissue in the TAA group. After NRL administration, SOD activities increased to 68.53±11.54 kU/mg protein tissue in the TAA+ NRL group. Mean kidney tissue GPx levels were 0.12±0.03 U/mg protein tissue in the Control group. TAA administrated significantly decreased GPx levels to 0.06±0.04 U/mg protein tissue in the TAA group. After NRL administration, GPx levels significantly increased to 0.15±0.03 U/mg protein tissue in the TAA+ NRL group.

**Histopathological Results:**
In Control (Figure 1A, 4A) and NRL (Figure 1B, 4B) groups heart and kidney tissues were observed normal histological appearance. In control and NRL groups heart tissue showed a normal myofibrillar structure with striations, branched appearances, and continuity with adjacent myofibrils. In Control and NRL groups kidney tissue showed a normal tubular and glomerular structures. Cardiac muscle cells were also normal; their large purple nucleus was located in the center of their pink colored cytoplasm. In heart tissue of TAA group, we detected hemorrhage (Figure 2A, B, C, F), necrosis (Figure 2B), eosinophilic stained and pyknotic nuclei cells (Figure 2C), oedema and vacuolisation (Figure 2D), vascular congestion (Figure 2E), mononuclear cell infiltration (Figure 2F).

In kidney tissue of TAA group, we detected inflammatory cell infiltration (Figure 5A, B, D), hemorrhage (Figure 5A, D, E), glomerular degeneration (Figure 5B,C,D), vascular congestion (Figure 5D), vacuolization of tubular epithelial cells and oedema between the tubules (Figure 5E), epithelial atrophy and cell desquamation in the tubules and casts in tubular lumina (Figure 5F).

Histopathological changes were more severe in the TAA group than in the group of treatment. On the other hand, histopathological damages decreased in the TAA + NRL (Figure 3, 6) group. NRL administration reduced TAA induced in comparison to compared to that of TAA treated groups for heart and kidney tissues. Histopathological scores are shown in Table 3.

**Discussion**
The reason for the toxic effect of TAA, which is used as a metal sulfide source, on tissues such as the heart, liver, kidney, and brain is the induction of oxidative stress. TAA-related toxicity is characterized by increased TBARS levels and increased ROS production. In our study, NRL treatment against TAA-induced heart and kidney toxicity was investigated in terms of antioxidant parameters and histopathological changes. TAA significantly increased TBARS levels in heart and kidney tissue and decreased antioxidant enzymes. Therefore, NRL treatment potentially decreased side effects of TAA and may be beneficial against these alterations.

Chemicals taken into the organism through environmental and industrial can cause oxidative stress. Compounds of natural origin can help reverse these effects. Natural antioxidant agents are thought to be beneficial in preventing imbalances in the antioxidant system that develop because of exposure to toxic substances.

Bioflavonoids are naturally obtained and have antioxidant, anti-inflammatory, and anti-apoptotic properties. Notably, they prevent multiple organ damage caused by oxidative stress and ROS. NRL shows antioxidant activity by eliminating free radical and reactive oxygen species. NRL can prevent lipid peroxidation by lowering TBARS levels. It also prevents the generation of hydroxyl radicals and reduces nitric oxide production. Thus, NRL has radical scavenging activity and is a good antioxidant against oxidative stress. In previous studies have shown that NRL exhibits strong antioxidant activity at the administration of 25, 50, and 75 mg/kg.
causes a considerable reduction in nitric oxide levels and significantly increases SOD and CAT levels. In this context, it protects cells from impairment due to oxidative damage by enhanced the generation of antioxidant enzymes.

**Changes in oxidative stress parameters**
The current different studies suggest that the most serious toxic effect of TAA was observed in the liver. TAA can act as an electrophilic agent and S-oxide groups formed by its metabolism attack biomolecules. Active metabolites due to TAA metabolism bind to cell lipids and proteins by oxidative stress, total antioxidant levels, and by binding them to cell lipids and proteins. Increased MDA levels indicate this toxicity. In previous studies, it was determined that, increased TBARS in environmental chemical agents rats result from enhanced membrane lipid peroxidation by free radicals and the failure of antioxidant defense mechanisms that prevent formation of excessive free radicals. In the same way, we found that TAA induced oxidative damage, increased TBARS levels, and decreased GSH levels and the activities of antioxidant enzymes, including SOD and CAT, in the heart and kidney.

In the current study, TAA induced nephrotoxicity by way of enhanced lipid peroxidation, decreased antioxidant enzyme system, and enhanced histopathological damage. Kidneys are highly vulnerable to damage from free radicals and oxidative stress from unsaturated fatty acids. Free radicals from TAA lead to severe kidney damage. Inflammation, oxidative damage, and apoptosis mechanisms are the most important factors causing renal function impairment. TAA exposure adversely affects the defense system, which prevents the accumulation of ROS. In another study on cardiac toxicity from TCDD, cardiac tissue had increased levels of TBARS. The parameters of the antioxidant enzyme activities decrease in the heart tissue due to xenobiotic exposure. Another study demonstrated that administration of environmental chemical agents to rats led to oxidative stress and was associated with significantly lower antioxidant activities of GSH, CAT and SOD. Thus, the present literature confirms our results. In this study, TAA toxicity decreased the activity of GSH, SOD, CAT, and GPx in kidney and heart tissues. No studies on heart damage caused by TAA exposure have been seen in the literature studies and we believe our study will be the first in this area. The another study demonstrated that TAA induced oxidative stress which approved by the decreases of serum SOD and GSH levels. These results obviously showed that TAA induced oxidative stress in experimental rats. Oxidative stress plays a primary role in the pathogenesis of multi organ toxicity. ROS are one of the main causes leading to the progression of pathophysiological changes of the organ injury. ROS formation occurs in an equilibrium created. However, when this balance is disrupted by external or internal xenobiotics, oxidative stress forms in the heart tissue. In vivo animal studies show that the resulting ROS lead to heart tissue damage. Another study demonstrated that administration of environmental chemical agents to rats leaded to oxidative stress and was associated with significantly lower antioxidant activities of GSH, CAT and SOD. Thus, the present literature confirms our results. In this study, TAA toxicity decreased the activity of GSH, SOD, CAT, and GPx in kidney and heart tissues. No studies on heart damage caused by TAA exposure have been seen in the literature studies and we believe our study will be the first in this area.

**Histopathological changes**
In different studies, histopathological effects from TAA indicate that the kidney cortex is more affected than the medulla. In the images of the light microscope, significant histopathological changes were observed as a result of the glomerular blockage, focal mesangial cell proliferation, increased accumulation of collagen in the renal medulla and fibrin. Thus, renal cell damage can occur as a decrease in tubules and glomerular filtration rate. Histopathologically, the toxic effects of TAA on the organs of experimental animals were investigated by several studies. These studies showed that the light microscopic examinations of renal and liver tissues revealed severe histopathological changes. In the study of Ciftci et al., TAA's tissue toxicity was examined and as part of our study, toxic effects on kidney and heart tissues were observed. Other studies have shown that nephrons of kidneys are negatively affected by nephrotoxic, xenobiotic, and heavy metal exposure. One of these adverse effects, a decrease in the functional mass of the kidneys can be shown, leading to faster cell deaths. In this context, kidney tissue is very vulnerable to such situations. In vivo studies are suggested that exposure to nephrotoxicants has caused considerable cellular damage. In the study of Edward et al., were determined renal tubular damage of kidneys TAA-induced as morphologically. The tubular damage is reversed with the *Vitex negundo* extract used in the study. The results of the another study, it was showed that the extract of basil leaves could be used as safe potential natural products in the treatment of TAA induced nephrotoxicity. The histopathological results of our study were found to be compatible with the literature. In our previous studies, it was determined that TCDD exposure caused histopathological changes in heart tissue including severe necrosis and bleeding. In our study, TAA has been shown to cause histopathological changes in the heart tissue, such as necrosis and bleeding compared with the control group. In another study, TAA was shown to cause histopathological damage to the reproductive system. We are detected histopathological changes decreased significantly with NRL treatment. In this study results are the first to determine that NRL therapy reverses the histopathological damage of TAA on the heart tissue. In our study, it has been shown that NRL treatment can make a significant contribution to the literature as it reduces oxidative damage to the heart tissue and prevents histopathological damage.
Conclusion
In conclusion, the present study shows that NRL can inhibit TAA-induced oxidative damage of the heart and kidneys. As a result, administration of TAA led to a significant increase in TBARS levels and a significant decrease in antioxidant system (SOD, CAT, GPx and GSH) activities, causing histological damage to the heart and kidney tissues. Thus, NRL may be useful as new pharmacological agent for ameliorating biochemical and histopathological damage in heart and kidney tissues.

Conflict of Interest: The authors have declared no conflict of interest.

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**Figure 1:** In Control (A) and NRL (B) groups; heart tissue showed normal histological appearance. A, B: H-E; X40.
Figure 2: In TAA group: hemorrhage (black asterisk) (A,B,C,F), necrosis (thick black arrows) (B), eosinophilic stained and pyknotic nuclei cells (white arrows) (C), (F), oedema (white asterisk) and vacuolisation (thin black arrows) (D), vascular congestion (E), mononuclear cell infiltration (thin black arrows) were observed in heart tissue. A:H-E; X10, B, F:H-E; X20, C, D, E: H-E; X40.
Figure 3: In the TAA + NRL group: heart tissue findings were decreased compared with the TAA group. Little hemorrhage (black asterisk) (A), a few eosinophilic stained and pyknotic nuclei cells (white arrows) (B), little mononuclear cell infiltration (thick black arrows) and vascular congestion (thin black arrow) (C) were observed in heart tissue. A, B, C: H-E; X40.
Figure 4: In Control (A) and NRL (B) groups; kidney tissue showed normal histological appearance. A, B: H-E; X40.
Figure 5: In TAA group; inflammatory cell infiltration (white asterisk) (A, B, D), hemorrhage (A, D, E), glomerular degeneration (black arrows) (B, C, D), vascular congestion (D), vacuolization of tubular epithelial cells (black arrows) and oedema between the tubules (white asterisk) (E), epithelial atrophy and cell desquamation in the tubules and casts in tubular lumen (F) were observed in kidney. A, B, C: H-E; X20, D, E, F: H-E; X40.
Figure 6: In the TAA+NRL group, kidney tissue findings decreased compared to the TAA group. We observed little inflammatory cell infiltration (white arrows) (A,B), casts in tubular lumen (white asterisk) (B) and a few vacuolization of tubular epithelial cells (B). A,B: H-E; X20, C: H-E; X40.

Table 1: The effect of nerolidol on oxidative stress markers of heart tissues in rats. Values are presented as means ± SD.

|       | TBARS (nmol/g tissue) | Reduced GSH (nmol/ml tissue) | CAT (k U/mg protein) | SOD (U/mg protein) | GPx (U/mg protein) |
|-------|-----------------------|-----------------------------|----------------------|--------------------|-------------------|
| Control | 11.49±3.43\textsuperscript{x} | 41.82±6.51\textsuperscript{y} | 0.302±0.10\textsuperscript{z} | 86.68±7.71\textsuperscript{z} | 0.55±0.09\textsuperscript{y} |
| TAA    | 17.42±1.64\textsuperscript{x} | 17.64±0.93\textsuperscript{y} | 0.004±0.0002\textsuperscript{z} | 66.98±9.32\textsuperscript{z} | 0.12±0.02\textsuperscript{y} |
| NRL    | 3.79±0.46\textsuperscript{x} | 49.38±6.78\textsuperscript{y} | 0.013±0.0002\textsuperscript{z} | 87.45±2.11\textsuperscript{z} | 0.39±0.01\textsuperscript{y} |
| TAA+ NRL | 8.44±0.28\textsuperscript{x} | 27.58±1.04\textsuperscript{y} | 0.009±0.003\textsuperscript{z} | 78.96±13.18\textsuperscript{y} | 0.18±0.06\textsuperscript{y} |

\textsuperscript{x,y,z} < 0.001
x: compared with Control Group, y: compared with TAA Group, z: compared with TAA+NRL Group.

Table 2: The effect of nerolidol on oxidative stress markers of kidney tissues in rats. Values are presented as means ± SD.

|       | TBARS | Reduced GSH | CAT | SOD | GPx |
|-------|-------|-------------|-----|-----|-----|
| Control |       |             |     |     |     |
| TAA    |       |             |     |     |     |
| NRL    |       |             |     |     |     |
| TAA+ NRL |       |             |     |     |     |

x: compared with Control Group, y: compared with TAA Group, z: compared with TAA+NRL Group.
Table 3. Comparison of the effect of NRL on histopathological damage caused by TAA in heart and kidney tissues.

| GROUPS   | Histopathologic score of heart | Histopathologic score of kidney |
|----------|--------------------------------|---------------------------------|
| Control  | 0,62 ± 0,10                    | 0,83 ± 0,08                     |
| TAA      | 2,40 ± 0,09<sup>a</sup>        | 2,10 ± 0,14<sup>a</sup>        |
| TAA + NRL| 1,30 ± 0,07<sup>b</sup>        | 1,45 ± 0,07<sup>b</sup>        |
| NRL      | 0,87 ± 0,10                    | 0,95 ± 0,13                     |

a: There is difference (p<0,0001) between TAA and the other groups.

b: There is difference (p<0,0001) between the TAA+NRL group and the other groups.

The mean differences the values bearing different superscript letters within the same column are statistically significant. (p<0,0001) (TAA: Thioacetamide, NRL: Nerolidol).

Tables Legends
Table 1: The effect of nerolidol on oxidative stress markers of heart tissues in rats. Values are presented as means ± SD.
Table 2: The effect of nerolidol on oxidative stress markers of kidney tissues in rats. Values are presented as means ± SD.
Table 3. Comparison of the effect of NRL on histopathological damage caused by TAA in heart and kidney tissues. (Arithmetic mean ± SE).