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# Investigation of the Effects of Hesperidin on Bisphenol-A Induced Neurotoxicity in Rats

## Ratlarda Bisfenol-A'nın Neden Olduğu Nörotoksisite Üzerine Hesperidinin Etkilerinin Araştırılması

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## ABSTRACT

Bisphenol A (BPA) is an adhesive substance used in the production of food packaging, electronic devices, dental sealants and polycarbonate plastics. This substance, which can leak into products during industrial processes, can be taken into the body through contact or consumption. BPA causes oxidative damage in the body and toxicity to organs. This study was conducted on 52 male rats. The rats were randomly distributed into 4 separate groups, with 13 animals in each. Experiment groups were formed as follows: Control: 1 ml of olive oil was administered intragastrically for 14 days. Hesperidin (HESP): HESP was administered intragastrically at a dose of 50 mg/kg for 14 days. BPA: BPA dissolved in olive oil was administered intragastrically at a dose of 100 mg/kg for 14 days. BPA+HESP: BPA at a dose of 100 mg/kg and HESP at a dose of 50 mg/kg were administered intragastrically for 14 days. Brain tissue samples from the rats were collected on the 15th day of the experiment while the rats were under sevoflurane anesthesia. Histopathological and biochemical analyzes were performed on the brain tissues of the rats. As a result of the study, it was observed that HESP had a protective effect on BPA-induced neurotoxicity in rats and triggered the antioxidant mechanism responsible for defense in the cell. It was opined that the degenerative and necrotic tissue damage caused by BPA in the brain tissue decreased with the effect of Hesperidin.

**Keywords:** Bisphenol A, hesperidin, MDA, neurotoxicity, rat

## ÖZ

Bisfenol A (BPA), gıda ambalajı, elektronik cihazlar, diş dolguları ve polikarbon plastiklerin üretiminde kullanılan yapışkan bir maddedir. Bu madde, endüstriyel işlemler sırasında ürünlere sızabilir ve temas veya tüketim yoluyla vücuda alınabilir. BPA, vücutta oksidatif hasara ve organlara toksisiteye neden olabilir. Bu çalışma, 52 erkek sıçan üzerinde gerçekleştirilmiştir. Sıçanlar, her birinde 13 hayvan bulunan 4 ayrı gruba rastgele dağıtılmıştır. Deney grupları şu şekilde oluşturuldu: Kontrol: 1 ml zeytinyağı, 14 gün boyunca intragastrik olarak uygulandı. BPA maruz kalan gruplara HESP dozunda 100 mg/kg intragastrik uygulama ile verildi. HESP: HESP, 50 mg/kg dozunda 14 gün boyunca intragastrik olarak uygulandı. BPA: BPA, zeytinyağında çözülmüş olarak 100 mg/kg dozunda 14 gün boyunca intragastrik olarak uygulandı. BPA+HESP: BPA, 100 mg/kg dozunda ve HESP, 50 mg/kg dozunda 14 gün boyunca intragastrik olarak uygulandı. Deneyin 15. gününde, sıçanlar sevofluran anestezisi altındayken sıçanlardan beyin dokusu örnekleri toplandı. Sıçanların beyin dokularında histopatolojik ve biyokimyasal analizler yapıldı. Çalışma sonucunda, HESP'nin BPA tarafından indüklenen nörotoksisite üzerinde koruyucu bir etkisi olduğu ve hücre savunmasından sorumlu antioksidan mekanizmayı tetiklediği gözlemlendi. BPA'nın beyin dokusunda neden olduğu dejeneratif ve nekrotik doku hasarının, HESP'nin etkisi ile azaldığı düşünülmüştür.

**Anahtar Kelimeler:** Bisfenol A, hesperidin, MDA, nörotoksisite, rat

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## INTRODUCTION

BPA is a widely used fabrication chemical that is a colorless, crystalline solid with a distinct phenolic odor. It is commonly used in the production of food packaging, electronic devices, building materials, dental filling materials, toys, plastic, and feeding bottles.[1-6] BPA has become a global environmental pollutant because of its widespread use in industrial production and contact with food products.[7,8] BPA is recognized as an endocrine disruptor due to its ability to disrupt the regular functioning of hormones within the body. By interfering with hormones, BPA has the potential to cause a range of health issues, including reproductive disorders, developmental problems, and metabolic disorders. This substance, taken into the body orally and through the respiratory tract, interacts with many receptors in the hormonal system and causes severe damage to the endocrine system. As a result of these damages, toxicities are observed in the brain, liver, and other organs.[9] It has been established that BPA can induce neuropsychological and behavioral disorders by crossing the blood-brain block.[10] Exposure to neurotoxic substances such as BPA leads to the oxidation of the protein and lipid structures of cells in the brain, resulting in the overproduction of reactive oxygen and nitrogen molecules (ROS and RNS, respectively).[11-13] The increase in oxygen radicals and RNS leads to the activation of apoptotic pathways, which result in cell death by damaging the cell's organelles, macromolecules, and membrane structure. Chronic exposure triggers an inflammatory process in the brain that contributes to neurodegenerative diseases. Against this damage, brain tissue has various antioxidant defense mechanisms created by different enzymes, such as superoxide dismutase (SOD). Glutathione in its reduced form (GSH) is one of the critical internal antioxidants found in brain tissue and possesses strong scavenging properties against hydroxyl radicals (OH.).[14] Alongside these protective mechanisms, researchers are working on alternative approaches to mitigate the current side effects.[15] An example of such an approach involves utilizing safe, low-side-effect, and readily available herbal antioxidants in the treatment process. In recent experimental studies, HESP, a compound in the flavonoid group found in citrus fruits, green tea, and some vegetables, has been investigated for its antioxidant effects to eliminate and treat the toxic effects of BPA.[16-20] It has been reported that this compound, frequently utilized in both industry and cosmetics, possesses numerous properties, including anticarcinogenic, antiallergic, neuroprotective, immunomodulatory, and anti-diabetic effects. The objective of this study was to investigate the protective effects of HESP in BPA-induced neurotoxicity in rats and to contribute to the literature in line with the data obtained.

## MATERIALS AND METHODS

### Chemicals

BPA (≥99%) (Cas No: 80-05-7) and HESP (Cas No: 520-26-3) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Malondialdehyde (MDA) (Cat No: SL0475Ra), Superoxide Dismutase (SOD) (Cat No: SL0664Ra), Glutathione (GSH) (Cat No: SL0998Ra), and Nitric Oxide (NO) (Cat No: SL0531Ra) commercial ELISA kits were purchased from SunLong Biotech Co.LTD.

### Animals

The study utilized experimental animals sourced from the Medical Experimental Research and Application Center at Atatürk University. We used 52 male Sprague Dawley rats with an average weight of 250-300 g (2.5-3 months old). The animals in the experimental groups had unrestricted access to meet their daily nutritional and water requirements. This study was approved by the Animal Ethics Committee of Animal Experiments of the Veterinary Faculty at Atatürk University (18.08.2022/182).

### Experimental Protocol

All rats were weighed, and by random assignment, four different experimental groups were formed, each consisting of 13 rats. Active substance applications were made to the experimental groups at 9:00 for 14 days. BPA application was made 1 hour after the HESP application. Experiment groups were formed as follows: Control: 1 ml of olive oil was administered intragastrically for 14 days. HESP: HESP was administered intragastrically at a dose of 50 mg/kg[21,22] for 14 days. BPA: BPA dissolved in olive oil was administered intragastrically at a dose of 100 mg/kg[22,23] for 14 days. BPA+HESP: BPA at a dose of 100 mg/kg and HESP at a dose of 50 mg/kg were administered intragastrically for 14 days.

### Collection and Homogenization of Brain Tissues

On the 15$^{th}$ day of the experiment, the rats were euthanized under sevoflurane anesthesia by decapitation. Tissues were stored at -80°C. Tissue sections taken in equal amounts from brain tissues on the analysis day were transferred to capped tubes. It was completed with 1.5 ml of PBS (Phosphate buffered saline) solution with a pH of 7.4. Tubes were placed in the homogenizer device to perform the homogenization process. Afterward, the tissues were homogenized for 80 seconds at 5,000 rpm, followed by centrifugation at 7,000 rpm for 5 minutes. The

resulting supernatants were then carefully transferred into clean tubes.

Assessment of MDA levels, NO quantity, SOD and GSH enzyme activities in brain tissue:

The necessary analyzes for the determination of MDA levels, NO quantity, SOD and GSH activities in rats were performed according to the protocol using commercial rat ELISA kits. In line with the data obtained, evaluations were made between the groups.

**Histopathological Examination**

Upon the culmination of the evaluation, brain tissue specimens underwent fixation in a meticulous 10% formaldehyde solution for a duration of 48 hours. Subsequently, they were delicately enshrined within paraffin blocks through the standard course of tissue processing. Precise sections, each measuring a mere 4 µm in thickness, were meticulously extracted from every block. These sections were then meticulously prepared for histopathological scrutiny through a nuanced staining process employing hematoxylin-eosin (HE). The resulting slides were subjected to intense examination utilizing a luminous microscope of unparalleled quality (Olympus BX 51, JAPAN). The assessments were conducted with a discerning eye, discerning the histopathological nuances, and categorizing the sections based on their distinct characteristics: absent (-), mild (+), moderate (++), or severe (+++).

**Statistical Analysis**

After the completion of the studies, the statistical analysis of more than two independent groups was conducted using one-way ANOVA within the SPSS 20.00 (IBM SPSS Corp., Armonk, NY, USA) statistical data software. Subsequently, Tukey test was utilized for the acquisition and evaluation of quantitative values. The resulting values were presented as mean ± standard error of the mean (± SEM), and statistical significance was defined as $P < .05$. For histopathological analyses Kruskal Wallis test was used for comparison between groups, and Mann Withney U test was used for comparison of paired groups.

**RESULTS**

**Biochemical Analyses**

This study investigated the possible effects of Hesperidin on BPA-induced neurotoxicity in rats. The administration of BPA resulted in a notable increase in the MDA levels within the brain tissues when compared to the control group ($P < .05$). HESP demonstrated a significant reduction in the BPA-induced increase of MDA ($P < .05$). The MDA level in the HESP group was observed to be comparable to that of the control group. (Figure 1).

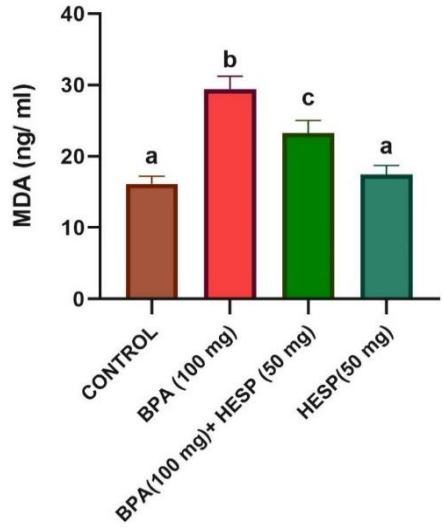


Figure 1. Representation of MDA levels in brain tissues taken from rats. Results were expressed as mean ± SD. Different letters indicate statistical differences between groups (P<0.05, n=8). SD: standard deviation

The introduction of BPA led to a pronounced decline in SOD activity within cerebral tissues, showcasing a marked difference from the control group ($P < .05$). Notably, HESP effectively thwarted the BPA-induced reduction in SOD activity, demonstrating a significant protective effect ($P < .05$). Furthermore, SOD activity levels in the HESP group were observed to closely mirror those of the control group, with no statistically significant difference noted ($P > .05$) (Figure 2).

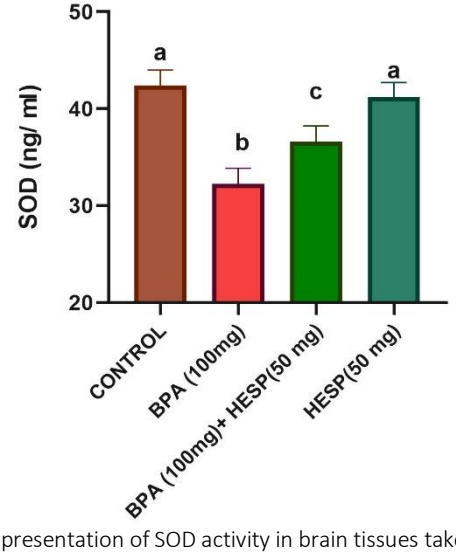


Figure 2. Representation of SOD activity in brain tissues taken from rats. Results were expressed as mean ± SD. Different letters indicate statistical differences between groups (P<0.05, n=8). SD: standard deviation

The administration of BPA markedly decreased the activity of glutathione (GSH) in brain tissues compared to the control group ($P < .05$). However, the detrimental effects of BPA on GSH activity were effectively alleviated by HESP ($P < .05$). Remarkably, the GSH activity in the HESP group did not exhibit a significant difference from those in the control group ($P > .05$) (Figure 3).

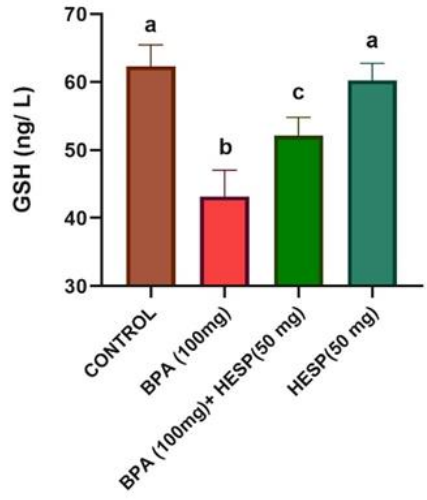


**Figure 3.** Representation of GSH activity in brain tissues taken from rats. Results were expressed as mean ± SD. Different letters indicate statistical differences between groups (P<0.05, n=8). SD: standard deviation

The administration of BPA led to a significant elevation in the NO levels within brain tissues compared to the control group ($P < .05$). HESP effectively mitigated the BPA-induced increase in NO ($P < .05$). Notably, the NO levels in the HESP group were comparable to those in the control group ($P > .05$) (Figure 4).

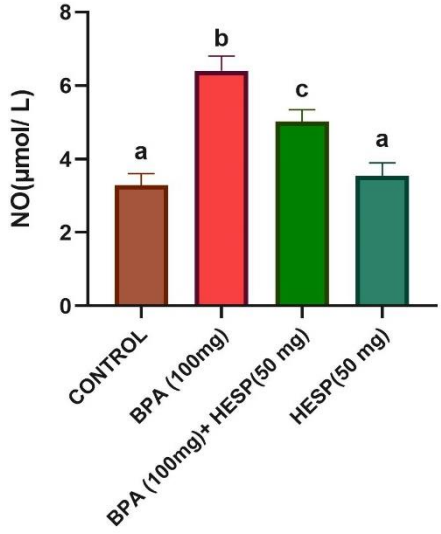


**Figure 4.** Representation of NO activity in brain tissues taken from rats. Results were expressed as mean ± SD. Different letters indicate statistical differences between groups (P<0.05, n=8). SD: standard deviation

**Histopathological Findings**

Control and HESP: When the brain tissues were examined histopathologically, normal histological appearance was detected.

BPA: Significant neuronal degeneration and necrosis, along with pronounced vascular hyperemia, were evident.

BPA+HESP: Moderate vascular hyperemia was noted, accompanied by moderate neuronal degeneration and mild necrosis (Figure. 5). A statistically significant difference was observed compared to the BPA group ($P < .05$). The histopathological findings are presented in Table 1.

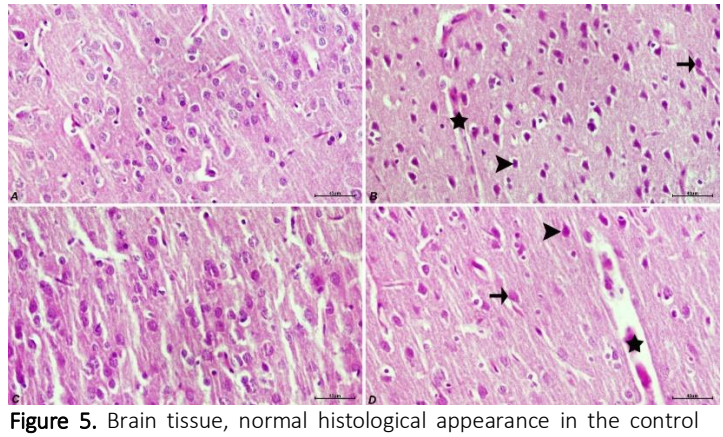

**Figure 5.** Brain tissue, normal histological appearance in the control group (A) and HESP group (C), degeneration (arrow) and necrosis (arrowhead) in BPA group (B) and BPA+HESP (D) group neurons, hyperemia in vessels (star), H&E, Bar: 40µm.

Table 1. Histopathological findings and scoring in brain tissue

| Groups | Degeneration in neurons | Necrosis in neurons | Hyperemia in the veins |
|---|---|---|---|
| Control | - | - | - |
| BPA | +++ | +++ | +++ |
| HESP | - | - | - |
| BPA+HESP | ++ | + | ++ |

(-): absent, (+): mild, (++): moderate, (+++): severe.

## DISCUSSION

BPA, widely used in the industrial field, is a substance that seriously harms health. Hence, individuals are consistently exposed to BPA in their everyday lives. Both in vivo and in vitro research have demonstrated the accumulation of BPA in various tissues, leading to the onset of diseases.[24-28] Research indicates that BPA induces oxidative stress, can

modify neurogenesis, and leads to neurological damage and cognitive disorders in organisms.[29,30] In this study, rats were selected to evaluate the existing complications in animals exposed to environmental toxic substances such as BPA. The objective was to evaluate HESP protective potential against BPA-induced neurotoxicity through biochemical and histopathological assessments.

HESP, a derivative of flavonoid, boasts various pharmacological advantages, notably encompassing significant anti-inflammatory and antioxidant properties. HESP has demonstrated noteworthy efficacy in alleviating inflammation, relieving pain, combating fungal and viral infections, and exhibiting potent antioxidant and anticancer activities.[31-33] Furthermore, recent studies indicate that HESP may be beneficial in neurodegenerative diseases, psychiatric disorders, demyelinating diseases, as well as ischemic-reperfusion injury and neuroinflammatory conditions.[34-36] Building upon these critical insights, we delved into investigating the potential protective effects of HESP against oxidative stress induced by BPA induced brain damage. This study signifies that HESP could play a crucial role in maintaining the health of brain tissue and mitigating the effects of oxidative stress.

The first indicator of cellular damage is the peroxidation of lipids in the cell membrane. MDA is one of lipid peroxidation's main products, reflecting the degree of membrane damage. A few studies have noted an elevation in MDA levels in specific rat tissues due to BPA exposure.[37,38] The study conducted by Abdou et al., investigates the neurotoxicity induced by BPA in male rats. The research reveals that administering specific doses of BPA to rats leads to a significant increase in the levels of MDA in brain tissues.[39] In the study conducted by Morsy et al., it was observed that the levels of MDA increased, indicating heightened oxidative stress in the hippocampus following exposure to BPA in rats.[40] These findings underscore the deleterious impact of BPA on hippocampal neurotoxicity and memory function. Our findings demonstrate that the application of HESP mitigated the increase in MDA levels induced by BPA-related neurotoxicity. Previous studies have found that HESP exhibits neuroprotective properties and supports the clearance of free radicals, leading to a reduction in lipid peroxidation products.[41-43] This is beneficial in terms of reducing oxidative stress.

An effective endogenous antioxidant defense mechanism neutralizes oxidative stress in the body.[44] Superoxide dismutase (SOD) is an essential endogenous antioxidant that reduces superoxide radicals in the cell and forms the first line of defense against oxidative damage. One of the antioxidants required for converting this harmful hydrogen peroxide into water and molecular oxygen is GSH. GSH, a crucial non-enzymatic antioxidant, is a tripeptide composed of cysteine, glycine amino acid, and glutamic acid. It is involved in the inhibition of lipid peroxidation. Some studies have shown that exposure to BPA reduces SOD and GSH activities in brain tissue. The research carried out by Ishtiaq and colleagues delves into the neurotoxic effects induced by BPA in male rats. The study uncovers that the administration of specific doses of BPA to rats results in a notable decrease in the activity of SOD and GSH in brain tissues.[45] Similarly, in a study conducted by T. Geetharathan on pregnant rats, a decrease in the activity of antioxidant enzymes SOD and GSH was observed in BPA-induced brain damage.[46] Consistent with previous research, our study compellingly demonstrates that the co-administration of hesperidin and BPA is significantly associated with an increase in SOD and GSH activities in the brain tissue of rats. On the other hand, studies have reported that HESP improves cognitive and motor functions by increasing the levels of antioxidant enzymes in the brain tissue and sera of rats.[41-43]

Nitric oxide (NO) reacts with the superoxide radical ($O2^{\cdot-}$) and turns into an oxidant factor called peroxynitrite. Peroxynitrite can react with the cell's DNA, lipid, and protein structures and inactivate the antioxidant forms GSH and GPx. Studies have shown that it increases NO activity due to BPA application in brain tissues. In the study conducted by Ayazgök and colleagues, it was observed that the exposure to BPA in SH-SY5Y neuroblastoma cells resulted in an increase in NO activity.[47] Furthermore, according to the study conducted by Xinyu Li and colleagues, it was observed that Hesperidin inhibits nitric oxide production in LPS-stimulated BV-2 microglial cells.[36] In alignment with these findings, research by Li C and the team demonstrated that Hesperidin suppresses nitric oxide production in the RAW246.7 macrophage cell line. These instances underscore the anti-inflammatory potential of Hesperidin in modulating nitric oxide levels in different immune cell types.[48] Consistent with prior research, our study convincingly demonstrates that the simultaneous application of HESP and BPA resulted in a significant reduction in NO levels in the brain tissue of rats.

It has been demonstrated in some experimental studies that BPA causes neuronal damage in brain tissue.[39,40] In this study, it was determined histopathologically that BPA caused severe degenerative and necrotic damage to

neurons in the brain tissue. It has been reported that HESP has protective activity against the toxic effects of BPA in experimental studies conducted in various tissues. It has been demonstrated by this study that Hesperidin, which is used against the neurotoxic effect of BPA, also protects the brain tissue against this toxic effect. As a result of the study, it was demonstrated histopathologically that HESP also has a protective effect in brain tissue against BPA toxicity.

In conclusion, this study was conducted to investigate the effects of HESP on BPA-induced neurotoxicity in brain tissues, some biochemical parameters and histopathological changes. With this research, it has been shown that Hesperidin successfully reverses BPA-induced changes in oxidative stress, changes in biochemical parameters, and inflammation. It has also been observed that Hesperidin prevents tissue damage by inhibiting lipid peroxidation and activating antioxidant enzymes.

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**Author Contributions:** Concept -M.B., F.Ç.; Design-M.B., F.Ç.; Supervision-M.B.; Resources- B.B.L.; Data Collection and/or Processing-M.B.; Analysis and/or Interpretation-M.B.; Literature Search-M.B.; Writing Manuscript-M.B., F.Ç.; Critical Review-M.B., F.Ç.

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