Curcumin Attenuates Nonylphenol-Induced Toxicity In Brain Development; An Experimental Study

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Abstract

Objective: Nonylphenol is an alkylphenol compound that has been widely used in the industry. It has endocrine-disrupting properties. The effect of alkylphenol compounds on development has been the subject of a limited number of studies. Herein, we aimed to examine curcumin's effect against nonylphenol toxicity on brain development.

Methods: For this study, 30 pregnant female Wistar albino rats from the Animal Laboratory of Erciyes University, Faculty of Medicine, were used. The rats were randomly divided into the following 5 groups; the control group, corn oil group (150μl/kg/day), nonylphenol group (50μl/kg/day), curcumin group (100mg/kg/day) and curcumin+nonylphenol group (100mg/kg/day+50 μl/kg/day). After the sacrification, histological and immunohistochemical evaluations were made.

Results: Histopathologically, vascular congestion, increased GFAP, and p-tau immunoreactivity intensity was found in the developing brain of the nonylphenol group. Moreover, co-treatment of nonylphenol administrated with curcumin showed slight pathological alterations with vascular congestion.

Conclusions: These data suggest that nonylphenol-induced increase in GFAP and p-tau immunoreactivity contributes to toxicity caused impairment in the rat brain. Additionally, curcumin had a neuroprotective effect against nonylphenol-induced neurotoxicity.

Keywords: Nonylphenol, Curcumin, GFAP, p-tau, brain development.

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INTRODUCTION

Nonylphenol (NP), an alkylphenol classified as an environmental endocrine disrupter, is widely present in the environment. The presence of nonylphenol in various nature compartments such as streams, sediments, soil,

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atmosphere, and biosphere has been shown. Nonylphenol is also used in industrial products such as pesticides, paints, and household toiletries [1]. The main concern about nonylphenol is the clustered concentrations on surface waters, mainly near wastewater lifting plants. NP's natural degradation in water is limited, and it is moderately soluble [2]. NP has been reported to induce various other adverse toxic effects on reproductive, immune, digestive, hemopoietic functions, and central nervous system (CNS) [3]. The CNS is highly sensitive to exogenous compounds. The possible potential nonylphenol effects on CNS have gained importance in recent years. As a lipophilic compound, NP can easily accumulate in the brain because of the immature blood-brain barrier in the early developmental stages [4]. Nonylphenol is a persistent organic pollutant, and a baby's CNS is more vulnerable to NP exposure than adults [5]. The brain is susceptible due to its structural components, such as unsaturated lipids and oxidative metabolism [6]. A few studies document that exposure to nonylphenol impairs the development and functioning of the brain, especially in the perinatal period, which is very important for CNS development) [3,4,7]. Many priorly conducted animal studies subjected the effects of NP on the reproductive system; however, only a few studies reported NP effect on the brains of the offspring in the experimental animals [8].

Natural plant products have been in use in many areas throughout the past. Curcumin, a polyphenolic phytochemical, has been used in the treatment of various diseases due to its featured pharmacological activities for centuries [9,10]. Curcumin, an active hydrophobic polyphenol, is extracted from the rhizomes of a herb called Curcuma Longa Linn of the Zingiberaceae family, in other words 'turmeric' [11]. Curcumin's anti-inflammatory, antioxidant, neuroprotective, and chemoprotective properties have been reported [12]. Studies conducted in rodents and humans have shown that curcumin can pass through the blood-brain barrier [13]. Curcumin is believed to exert neuroprotective effects against ischemic stroke and traumatic brain injury [14].

A growing body of evidence indicates that nonylphenol exposure in early life results in the impairment of the CNS; however, the fundamental mechanisms remain to be elucidated. The knowledge on the effect of toxic substances such as nonylphenol exposure on fetal brain development is limited. Herein, we aimed to investigate the toxicological features by focusing on the mischievous effects of NP on the normal development and function of the brain in vivo and to determine the possible protective effects of curcumin, which is known to have antioxidant properties, using immunohistochemical methods.

MATERIAL AND METHODS

Animal protocol

The ethics committee approval of the study was obtained from The Ethical Committee of Animal Experiments of Erciyes University with decision number 16/137. All the procedures performed at each stage of the study were conducted under the rules specified in the ethics committee directive. The minimum number of rats and fetuses was used according to the ethical rules of animal use and experimental design. Thirty adult female Wistar rats weighing between 200 to 250 g were used. Female rats were impregnated with male rats. Two female rats were placed in a cage (polypropylene steel wire mesh cages) with a male rat for the purpose from 5 p.m. to 8 a.m. The female rats with a sperm-positive vaginal smear were accepted to be on day 0.5 of pregnancy and were housed in individual cages under a controlled temperature of 21 ± 3 ˚C with a regular photoperiod (12-h light/12-h dark cycle). The rats were fed ad libitum with a boosting meal (granule pellet containing 21% raw protein). The consumed water of the rats was provided by tap water and was controlled and changed daily.

Experimental groups design

In the present study, rats considered as 0.5 days pregnant were divided into five groups randomly as follows:

1. Control group (n = 6): the group that was given standard food and water;
2. Corn oil group (n = 6): 50 μl /kg/day corn oil gavage was administered between the 5th and 20th days of pregnancy once a day;
3. Nonylphenol group (n = 6): 50 μl /kg/day nonylphenol dissolved in 100 μl corn oil was prepared daily (total 150 μl) was given via gavage between the 5th and 20th days of pregnancy once a day.
4. Curcumin group (n = 6): 100 mg/kg/day dose of

curcumin dissolved fresh daily in 150 μl corn oil (total 150 μl) was given via gavage between the 5th and 20th days of pregnancy once a day.

5. Curcumin+nonylphenol group (n = 6): Half an hour after the application of curcumin at a dose of 100 mg/kg/day and 50 μl dose of freshly prepared nonylphenol (a total 150 μl) was given via gavage between the 5th and 20th days of pregnancy once a day.

This current study's focus was fetal brain development. In order to examine the facts, nonylphenol gavage was given to the pregnant rats between the 5th and 20th days of their pregnancy. The experiment was initiated at the same time for all the groups. The pregnant rats were anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg) for cesarean operation on day 20. The abdominal area was cleaned with a 70% alcohol solution and opened with a transverse incision. The uteruses with the fetuses were dissected. In each group, 18 of the fetus's brains were used for the hematoxylin-eosin staining (H&E) and immunohistochemical staining (IHC) methods. The brain tissues of each fetus were fixated with formalin solution for histological examination.

Histological analysis

After the pregnant rats were sacrificed on the 20th day of their pregnancy and their offsprings were removed by cesarean section, excised offspring brain tissues were stored in 10% formaldehyde for fixation. The brain tissues were kept in fixation solution for 72 hours and then washed with running tap water. Following the washing process, increasing concentrations of alcohol was used to dehydrate the tissues via the immersion method. The clearance of the preparations was provided by multiple xylene washes. Then the train tissue blocks were embedded in paraffin wax. Sections were cut from the paraffin blocks with a thickness of 5 μm and mounted on polylysine-coated slides. Sections were deparaffinized by heating in a dry oven at 55°C-60°C for 120 minutes and then immersed in fresh xylene for 10 minutes for the third time. Rehydration of the sections was provided by immersion in decreasing concentrations of alcohol. Hematoxylin (Merck; Darmstadt, Germany) and eosin (Merck; Darmstadt, Germany) were used to stain the sections were stained in order to examine the general histological structure via a light microscope (Olympus BX51, Tokyo, Japan). All preparations were examined under this microscope, and images representing the groups were obtained.

Immunohistochemical analysis

The immunohistochemical examination was performed with the avidin-biotin-peroxidase technique to analyze p-tau and GFAP (Anti-Glial Fibrillary Acidic Protein) expressions. In this study, the immunoreactivity intensity was analyzed in the brain tissue of fetuses in all groups. Cross-sections of the tissue blocks with a 5 μm thickness were incubated at 60°C. In order to accomplish deparaffinization, the tissues were rinsed with sequential alcohol solutions, and distilled water was used to extract the alcohol. A staining kit (Thermo Fisher Scientific, USA) was used for the following steps. The surfaces of the tissues in the preparations were incubated overnight by dripping p-Tau rabbit polyclonal primary antibody from Santa Cruz (CA) and GFAP mouse monoclonal primary antibody from Millipore (USA). After the completion of the washing process, a biotin-secondary antibody was used to incubate the sections for 15 min followed by the repetition of the washing process. After the streptavidin peroxidase exposure for 15 min, the sections were exposed to the substrate for 5 min to make immunoreactivity apparent. Following this procedure, the sections were washed with deionized H_2O for 5 min. As the last step, chromagen DAB containing diaminobenzidine substrate was added to the environment, and the immune reaction was allowed to take place for approximately 5-10 minutes. Mayer's hematoxylin was used as the background stain. The prepared sections were examined by using an Olympus BX51 microscope (Tokyo, Japan). Photographs were taken from IHC applied preparations by using the same microscope. Five random fields were observed from each group's preparations. The photographs taken were transferred to the computer. Expression measurements of p-tau and GFAP were performed on the obtained photographs via ImageJ.

Statistical analysis

The data were represented as mean ± SEM (standard error of the mean), and the statistical analysis was performed by using GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA). The comparison of two groups in multiple groups was performed using one-way ANOVA

with Bonferroni analysis. A p-value of <0.05 was considered statistically significant.

RESULTS

Histologic findings

For histological evaluation, the hematoxylin-eosin staining was used to visualize the nucleus and cytoplasm of cells. Under H&E staining, the nucleus and cytoplasm had gained a blue and pink color, respectively, as shown in Fig. 1. Examined brain sections present normal histologic structure with the cell bodies of nerves in control rats. In spite of this, pathological changes in vessels were detected in the developing brain of the nonylphenol group compared with the control group. Microscopic examination of the brain showed vascular congestion in both gray and white matter. The brain tissues from only corn oil and the curcumin-treated group showed normal structure. Moreover, co-treatment of nonylphenol administrated with curcumin showed little pathological alterations with vascular congestion when compared with only the nonylphenol group (Fig. 1).

Immunohistochemical findings

Nonylphenol increases damage in the brain, though to a relatively small extent. Figure 2 presents these results and, in addition, shows the effects of curcumin treatment on both healthy and nonylphenol-induced rats. The activation of astrocyte upregulation is associated with neuronal cell death. GFAP expression was remarkably high in NP treated group. An apparent reduction in GFAP expression was observed in the curcumin co-treated group compared to the NP group. Expression of GFAP was negligible in control animals.

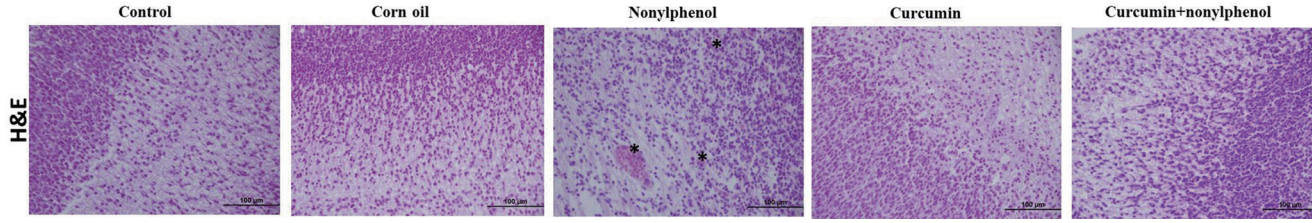

Fig. 1. Photomicrographs of the brain in cross-section were taken in all experimental groups. The normal brain tissue structure in the control group. The damaged brain structure after nonylphenol exposure. The nonylphenol+curcumin group's histological images revealed a significant improvement in brain histostructure. (H&E staining), original magnification, ×40. Star; vascular congestion.

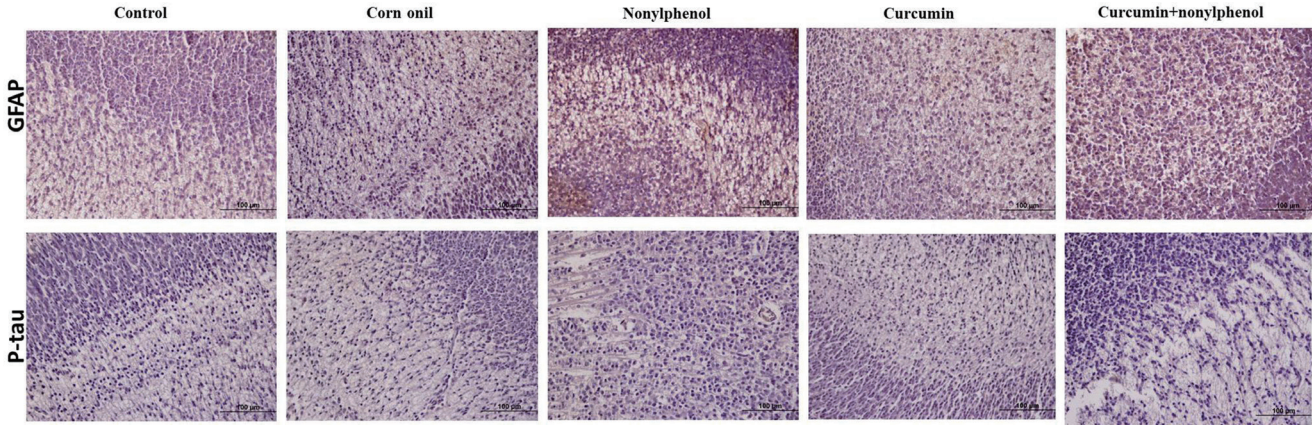

Fig. 2. The photomicrograph shows representative frontal brain sections of control, corn oil, NP, curcumin, and NP+curcumin stained for GFAP. A profound expression of the GFAP was observed in the NP group as compared to the control group. Curcumin administration remarkably decreased the expression of GFAP as compared to the NP-alone group.

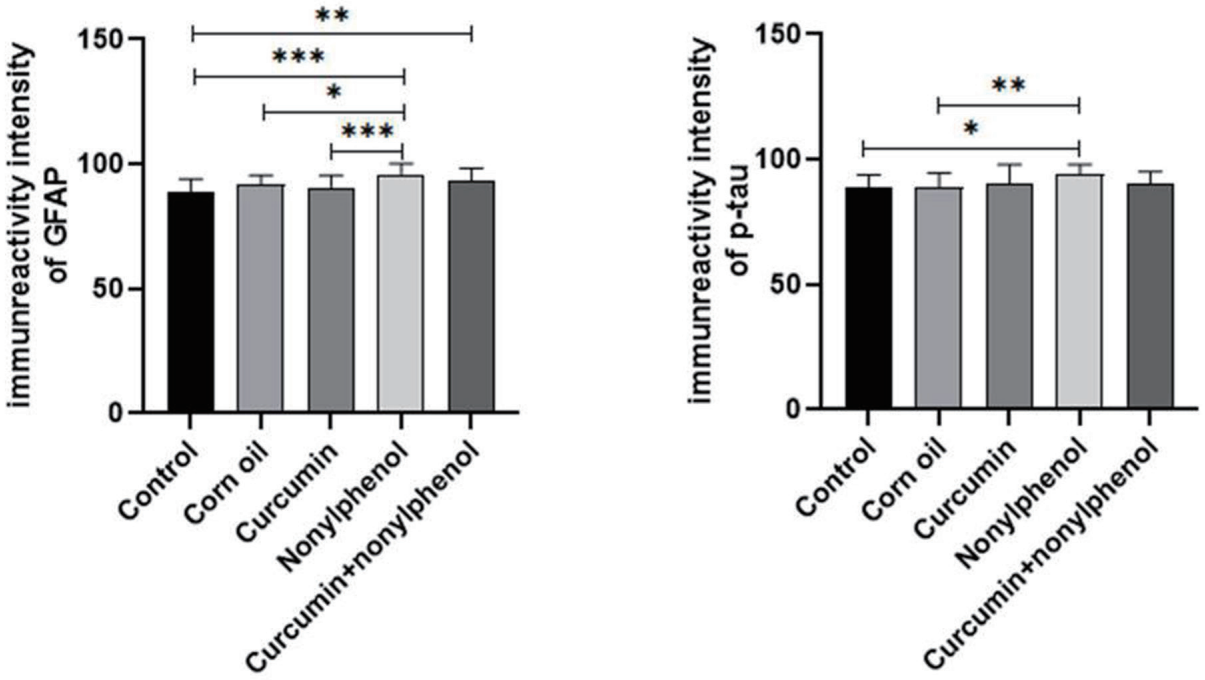

Fig. 3. GFAP and p-tau immunoreactivity intensity results. The values are presented as means±standard deviations. *p<0.05, ** p<0.01, *** p<0.001

Furthermore, we have investigated the influence of NP on the p-tau, a critical factor in neurodegenerative diseases, immunoreactivity in each experimental group. In order to verify NP-induced Tau hyperphosphorylation, we performed immunohistochemical analysis in the brain. In the nonylphenol-administrated group, p-tau expression increased compared to all other groups. P-tau immunoreactivity intensity was significantly increased in nonylphenol brain tissues compared with the control rats. No significant difference was observed in the p-tau immunoreactivity intensity among control, corn oil, curcumin and curcumin+nonylphenol groups (Fig.2).

DISCUSSION

Alkylphenol compounds are the member of a variety of synthetic environmental estrogen compounds and are widely present in the environment due to industrial discharge [1]. Nonylphenol is a typical alkylphenol compound resulting from the industrial discharge and environmental estrogens, with a detrimental feature. Herein, the administration of nonylphenol resulted in severe toxic events in the offsprings of rats.

Humans are exposed to nonylphenol from contaminated drinking water, food, breast milk, food containers, and personal care products [15]. Notably, nonylphenol has been detected in various human samples, such as urine, breast milk, adipose tissue, and fetal cord blood [16]. Gestation is a critical period of neurodevelopment, and any damage affecting CNS during this period may severely impair the cognitive function, intelligence, motor, and social function [17]. The CNS is vulnerable to environmental pollutants, and the adverse effects of nonylphenol on the central nervous system have become a hotspot of research. Exposure to nonylphenol is hypothesized to impair the CNS and cause cognition, attention, and motor dysfunction [18]. Priorly, a step-down avoidance test was examined in rats exposed to maternal nonylphenol, and learning and memory ability impairment was observed [19]. Nonylphenol exposure of the fetuses may have been through transplacental absorption. The vulnerability of the brains to nonylphenol neurotoxicity is

due to the immature blood-brain barrier. In line with the previous sentence, exposure to nonylphenol during the critical periods of brain development irreversibly harmed offspring rats in adolescence or adulthood [20].

In various areas, natural plant products have been in use throughout the past [9]. Curcumin is a polyphenolic phytochemical derived from Indian dietary fiber spices. It has been used in the treatment of various diseases due to its wide-ranging pharmacological activities for centuries [10]. Strikingly, curcumin has been reported as non-toxic and safe for clinical applications in the conducted researches [21]. Curcumin's pivotal role in the regulation of cell differentiation has been the subject of many studies recently. Curcumin could strongly affect the proliferation and differentiation of the neural progenitor cells and the generation, synaptogenesis, and migration of effective nerves [22]. Curcumin studies are increasing to explore curcumin's various therapeutic features, including analgesic, antioxidant, anti-inflammatory, and antimicrobial activities [23].

In this paper, we examined curcumin's role as a potential exclusive prophylactic antioxidative agent in nonylphenol-induced neurotoxicity in the rat model. The animal model was established by maternal nonylphenol gavage during pregnancy. Histopathologically, there were no morphological changes in the brain of the curcumin given animals. The frontal brain regions appeared normal. No neuronal damage was present. There were no glial cell changes, but vascular congestion presence was found in the NP group. In the nonylphenol-induced group, hyperchromatic cells, neuronal eosinophilia, nuclear pyknosis, and neuronal karyorrhexis were observed. Previous studies have reported similar changes [7]. These toxic effects of NP were restrained by curcumin pre-treatment, highlighting the neuroprotective action of curcumin.

GFAP's enhanced expression is used as a biomarker indicating gliosis which occurs due to sensitive and specific indices of toxicant and disease-induced neural damage. GFAP expression may be affected by age, neuronal damage, and sex steroid hormones [24]. Tau pathology is a common feature of several neurodegenerative disorders, together known as tauopathies. Phosphorylated tau protein (p-tau), an intracellular, microtubule-associated protein, is highly enriched in axons. Hyperphosphorylation and pathological aggregation of Tau indicate an axonal injury and are a common feature of many neurodegenerative diseases with axonal degeneration [25]. Therefore these observations, supporting the hypothesis, provide strong evidence of nonylphenol-induced injury in our study.

CONCLUSION

In conclusion, our study has demonstrated the neurotoxic actions produced by nonylphenol exposure in gestation and the protective role of curcumin in ameliorating the negative effects of nonylphenol in the rat brain. We assume that GFAP and p-tau immunoreactivity increase induced by nonylphenol causes a toxicological perturbation in the rat brain. The definite cellular mechanisms need further examination; however, according to our results, curcumin has an essentially neuroprotective effect against nonylphenol-induced neurotoxicity. This being the case, its potential preventive application to forestall endocrine-disrupting chemicals' harmful effects should be further considered.

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Ethics approval and consent to participate

This study was approved by the Ethical Committee of Animal Experiments of Erciyes University (16/137).

Competing interests

The authors declare that there is no actual or potential conflict of interest in relation to this article.

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