Research in Pharmaceutical Sciences, December 2022; 17(6): 677-685
Received: 30-06-2022
Peer Reviewed: 13-08-2022
Revised: 05-09-2022
Accepted: 02-10-2022
Published: 29-10-2022

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Aflatoxin G1 exposure altered the expression of BDNF and GFAP, histopathological of brain tissue, and oxidative stress factors in male rats

Toraj Zamir-Nasta[1], Ardeshir Abbasi[2], Seyran Kakebaraie[1], Arash Ahmadi[3], Mona Pazhouhi[1], and Cyrus Jalili[1,*]

[1]Medical Biology Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, I.R. Iran.
[2]Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, I.R. Iran.
[3]Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, I.R. Iran.

Abstract

Background and purpose: Aflatoxins are highly toxic compounds that can cause acute and chronic toxicity in humans and animals. This study aimed to evaluate the expression of BDNF and GFAP, histopathological changes, and oxidative stress factors in brain tissue exposed to aflatoxin G1 (AFG1) in male rats.
Experimental approach: Twenty-eight male Wistar rats were used. Animals were randomly divided into 4 groups of 7 each. The control group received 0.2 mL of corn oil and the treatment groups were exposed to AFG1 (2 mg/kg) intra-peritoneally for 15, 28, and 45 days. The tissue was used for histopathological studies, and the level of TAC, SOD, and MDA, and the expression of BDNF and GFAP genes were evaluated.
Findings/Results: Real-time PCR results showed that AFG1 increased GFAP expression and decreased BDNF expression in AFG1-treated groups compared to the control group. The tissue level of TAC and SOD over time in the groups receiving AFG1 significantly decreased and the tissue level of MDA increased compared to the control group. Histopathological results showed that AFG1 can cause cell necrosis, a reduction of the normal cells number in the hippocampal region of CA1, cerebral edema, shrinkage of nerve cells, formation of space around neuroglia, and diffusion of gliosis in the cerebral cortex after 45 days.
Conclusion and implication: AFG1, by causing pathological complications in cortical tissue, was able to affect the exacerbation of nerve tissue damage and thus pave the way for future neurological diseases.

Keywords: Aflatoxin G1; BDNF; Brain tissue; GFAP.

INTRODUCTION

Aflatoxins (AFs) are mycotoxins generally produced by the fungus *Aspergillus flavus* which is a common contaminant of food items such as corn, spices, rice, nuts, and flour that can cause acute and chronic toxicity in humans and animals. So far, different types of AFs have been identified, including AFB1, AFB2, AFG1, and AFG2 (1-3). AFG1 is one of the most common sources of contamination in cereals and foodstuffs in northern China, which has the highest incidence of lung and esophageal cancer (4). It can be metabolically activated by CYP2A13 and dramatically reduces biological cell function, increases cell apoptosis, and prevents cell cycle continuation and subsequent DNA damage (5). Neurodegenerative diseases are a group of acute or chronic diseases, depending on the reason or condition, that cause the destruction of neurons and glial cells in different parts of the brain and spinal cord. Reports of the effects of AFs on the nervous system have been shown to cause several pathological disorders, including hypothalamic neuropeptide dysfunction, dopaminergic pathway dysfunction, anterior cortical thickness, and hippocampal pyramidal cell complication such as necrosis (6-9).

*Corresponding author: C. Jalili
Tel: +98-9188317220, Fax: +98-8334276477
Email: cjalili@yahoo.com

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.359434

This highly lipophilic mycotoxin could easily enter the cells and thus be metabolized to a potent oxidant molecule (AFB1-8,9-epoxide) and trigger oxidative stress (10). Therefore, after exposure to AFB1, simply with, *e.g.* consuming a contaminated meal, it easily enters the bloodstream and could penetrate through the blood-brain barrier into the brain and cause damage to the resident cells (9,11). Neurotrophins are important proteins that affect the growth, proliferation, survival, differentiation, and death of cells and are essential for maintaining a healthy nervous system. Among these, brain-derived neurotrophic factors (BDNFs) are of great interest because of their important role in synaptic plasticity, memory, and neurogenesis (12-14). Studies have shown that decreased BDNF gene expression causes problems with synapse function, resulting in decreased memory and Alzheimer's disease (15). Glial fibrillary acidic protein (GFAP) is a major cytoskeletal protein of glial filaments found in astrocytes (16).

Studies have shown that various toxins affect astrocyte function and GFAP expression (17,18). In a previous study, we showed that chronic exposure to AFG1 can impair spermatogenesis in testicular tissue by disrupting the apoptosis process (19). Due to the lack of research on AFG1 poisoning in brain tissue and its association with neurodegenerative diseases, this study aimed to evaluate the expression of BDNF and GFAP, histopathological changes in brain tissue, and oxidative stress factors of AFG1 exposure in male rats.

MATERIALS AND METHODS

Animals

In this *in vivo* study, 28 male Wistar rats (mean weight 230 g) were used. Rats were randomly grouped into 4 groups, 7 each. The control group received 0.2 mL of corn oil and the treatment groups were exposed to AFG1 (2 mg/kg) by intraperitoneal injection for 15, 28, and 45 days (20,21). Subsequently, the rats were sacrificed using ketamine-xylazine under deep anesthesia and brain tissue was removed under sterile conditions. All animal procedures performed in this study were in accordance with the ethical guidelines of Kermanshah University of Medical Sciences (Ethic No. IR.KUMS.MED.REC.1401.023).

Chemicals

AFG1 was purchased from Sigma Co (USA; Cas No. 1162-39-5). Primary antibodies were rabbit anti-mouse for GFAP (Biocare, USA, Cas NO: SKU065) and BDNF (Creative Biolabs, USA, Cas NO: CBMAB-AP613LY). Secondary antibody and 3, 3′-diaminobenzidine tetrahydrochloride (DAB) chromogen kit were purchased from Life Teb Gen Co. (Tehran, Iran). The total RNA was extracted using a Trizol kit (Sigma-Aldrich; MRC, USA).

Histopathological analysis

Tissue samples were fixed in formalin for 48 h. Formalin-fixed brain tissues were exposed to various ascending alcohols (70%, 80%, 90%, and two substitutions in absolute alcohols) and subsequently infiltrated and embedded with paraffin wax. All blocks of fixed tissues were stored at room temperature in a dark place until use. Histopathological sections (5 μm thick) were obtained using a rotating microtome (MICROM model, Germany, Serial: 21074) and collected on polylysine-coated slides. The slides were oven-dried and deparaffinized with xylene and hydrated through a graded series of ethanol (100%, 95%, and 80% ethanol) and distilled water. One section of each tissue was routinely stained with hematoxylin and eosin (H&E) and cresyl violet (22) for basic morphological evaluation.

Malondialdehyde assessment

Malondialdehyde (MDA) levels were assessed as an indicator of oxidative stress. Using a homogeneous buffer containing 5.55% KCl solution, the samples were standardized and then centrifuged at 1500 rpm for 10 min. Homogenized samples were added to the reaction mixture containing sodium dodecyl sulfate, acetic acid (pH 4.5), and thiobarbituric acid. The mixture was boiled for 30 min at 85 °C and the adsorption rate was measured using a spectrophotometer at 450 nm.

Table 1. Nucleotide sequences of primers.

Primer	Sense 5'-3'	Anti-Sense 5'-3'
GFAP	AGTAACATGCAAGAGACAGAGG	CTCCAGATCGCAGGTCAAG
BDNF	CTGGGTTAACTTTGGGAAATGC	CCTTCATGCAACCGAAGTATG
GAPDH	GTGAACCACGAGAAATATGACAAC	AGTGATGGCATGGACTGTG

Superoxide dismutase assessment

According to the protocol of the superoxide dismutase (SOD) assay kit (Bioengineering, Nanjing Jiancheng, China), the brains were homogenized using lysis buffer (1 mM Na_2EDTA, 150 mM NaCl, 10 mM PMSF, 10 mM tris, 1 mg aprotinin). Measurement of the SOD activity of the supernatant was possible by determining the reduction of nitro blue tetrazolium (NBT) following the production of O_2- from the xanthine-xanthinoxyase system. A unit of SOD was defined as the amount of protein that inhibits NBT reduction by 50%. Results were expressed as protein U/mg.

Total antioxidant capacity assessment

To evaluate the level of total antioxidants Randox kit and COBAS-MIRA plus device (manufactured by Roche, Swiss) were used according to the instructions. Results were expressed as protein U/mg.

Immunohistochemically evaluation of GFAP and BDNF proteins

Tissue sections were heated at 60 °C for 25 min and deparaffinized in xylene and rehydrated in graded alcohol. The tissues were pretreated with 10 mM citrate buffer for 10 min for antigenic retrieval. Staining was performed following the manufacturer's instructions (Biocare and ScyTek, USA). The slides were washed with phosphate-buffered saline (PBS) and blocked with peroxidase blocking solution (0.03% hydrogen peroxide containing sodium acid) for 5 min. Sections were incubated for 2 h at 4 °C in a humidified chamber with one of the GFAP (GFAP-α; Biocare, USA, Cas No. SKU065) and BDNF (Creative Biolabs, USA, Cas. No. CBMAB-AP613LY) primary antibodies. The sections were incubated with a sufficient amount of streptavidin conjugated to horseradish peroxidase in PBS containing an antimicrobial agent for 15 min. To visualize the reaction, slides were incubated for 10 min DAB. The slides were counterstained with Mayer's hematoxylin then dehydrated and mounted. The BDNF and GFAP-positive cells were counted in 1 mm^2 of the brain cortex. Moreover, the cellular distribution was evaluated by software.

Real-time polymerase chain reaction

To evaluate the expression of BDNF and GFAP (GFAP-α) genes by real-time polymerase chain reaction (RT-PCR), we used BDNF and GFAP primers as well as GAPDH primer as control (*Table 1*). The total RNA was extracted using TRIzol*TM* and chloroform method and the quantity and quality of the purified RNAs were verified by Nano-drop spectrophotometer and electrophoresis (*ThermoFisher Scientific*, USA) using 1% agarose gel, respectively. Complementary DNA (cDNA) synthesis was carried out by taking 1 μg RNA using a cDNA synthesis kit (Vivantis Technologies, Selangor DE, Malaysia) according to the manufacturer's protocol and RT-PCR was done as described earlier.

Statistical analysis

Data are presented as mean ± SD of three independent experiments. The treatment groups were compared to the untreated control group using one-way ANOVA followed by Tukey's posthoc test. *P*-values < 0.05 were considered statistically significant.

RESULTS

Histopathological study

In histopathological examination, different degrees of lesions and injuries were observed in the experimental groups (15, 28, and 45 days) compared to the control group. Histopathological results showed that AFG1 dilated perivascular space (cerebral edema) and shrieked neurons and space around neuroglia in cortical tissue in the 15-day group. Diffuse gliosis and necrotic necrosis were also observed

in the 28-day group and edema in the perivascular and cerebral parenchyma with hyperemia was observed in the 45-day group affected by AFG1 (Fig. 1). Cell necrosis in the hippocampal region of CA1 was observed in AFG1-receiving groups on days 15, 28, and 45. The cell is wrinkled (with a pycnotic nucleus) and without a definite range compared with the control group neurons. As can be seen in the pictures, the number of normal cells in the AFG1-receiving groups (15, 28, and 45 days) showed a significant decrease compared to the control group (Fig. 2).

Total antioxidant capacity level

Our results showed that the tissue level of total antioxidant capacity (TAC) over time in the groups receiving AFG1 (15, 28, and 45 days) had a significant decrease compared to the control group (Fig. 3A).

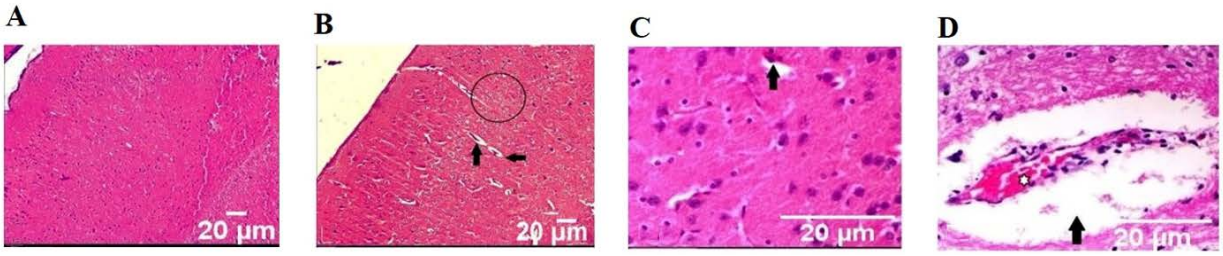

Fig. 1. Brain, (A) normal cerebral cortex tissue in one head of the control group; (B) the black arrows show dilatation of the perivascular space (cerebral edema) and the intracellular region of an example of nerve cell contraction and the formation of space around the neuroglia in the aflatoxin G1 group on day 15; (C) diffuse gliosis and single neuron necrosis (black arrow) in a field clone challenged with aflatoxin G1 on day 28 after inoculation; (D) edema in the perivascular space and brain parenchyma (black arrow) with hyperemia (white star) in the aflatoxin G1 group on day 45. (10X, 40X H&E staining).

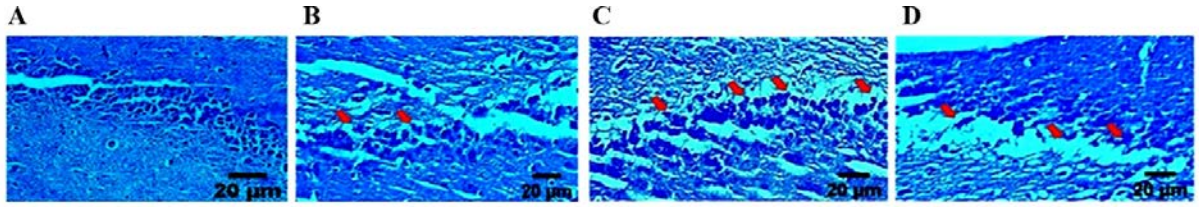

Fig. 2. (A) Control group; (B) 15 days after receiving aflatoxin G1; (C) 28 days after receiving aflatoxin G1, and (D) 45 days after receiving aflatoxin G1. Arrows indicate damaged cells in the hippocampal region of CA1 in groups receiving chronically aflatoxin that the shape of the cell is altered (the nucleus of pyknosis) and without a definite range compared to neurons in a control group. As can be seen, the number of normal cells in the 15, 28, and 45 groups receiving aflatoxin G1 showed a significant decrease compared to the control group.

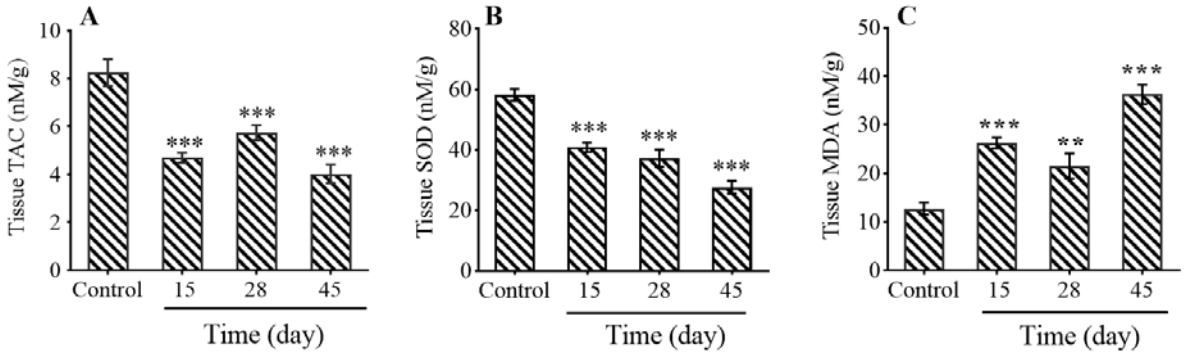

Fig. 3. Percentage of (A) TAC, (B) SOD, and (C) MDA changes in different groups receiving aflatoxin G1 at 2 mg/kg for 15, 28, and 45 days. Data are presented as mean ± SD. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate significant differences compared to the control group. TAC, Total antioxidant capacity; SOD, superoxide dismutase; MDA, malondialdehyde.

SOD and MDA level

The tissue level of SOD over time in the groups receiving AFG1 (15, 28, 45 days) had a significant decrease compared to the control group (Fig. 3B). Also, the tissue level of MDA over time in the groups receiving AFG1 (15, 28, 45 days) had a significant increase compared to the control group (Fig. 3C).

GFAP and BDNF expression

GFAP expression in the cortical cell line of brain tissue in the groups that received AFG1 (15, 28, 45 days) was increased significantly compared to the control group (Fig. 4A). Immunohistochemical studies of GFAP protein also showed that the increased rate of synthesis of this protein over time in the cortex of AFG1-receiving groups (15, 28, 45 days) compared to the control group (Fig. 5).

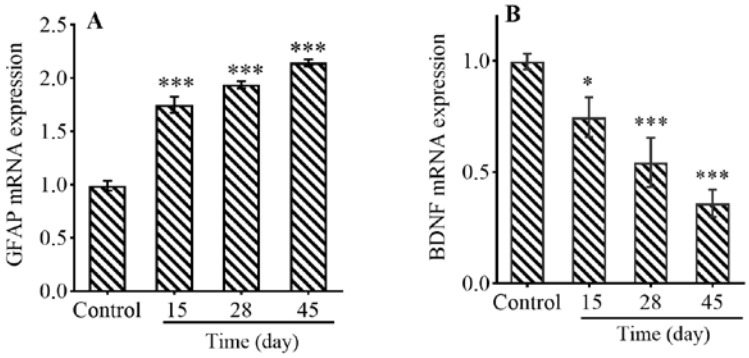

Fig. 4. Quantitative evaluation of (A) GFAP and (B) BDNF gene expression based on GAPDH. Different groups received aflatoxin G1 at 2 mg/kg for 15, 28, and 45 days. Data are presented as mean ± SD. **P* < 0.05 and ****P* < 0.001 indicate significant differences compared to the control group. GFAP, Glial fibrillary acidic protein; BDNF, brain-derived neurotrophic factors.

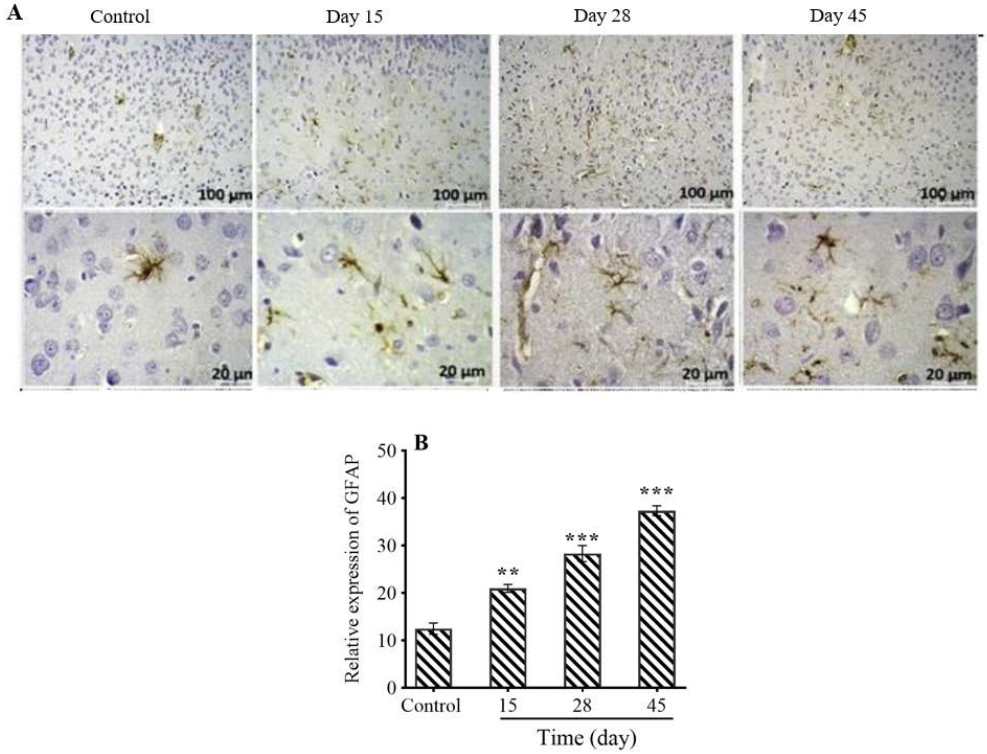

Fig. 5. (A) Immunohistochemical staining of the cerebral cortex of the rats receiving aflatoxin G1 at 2 mg/kg for 15, 28, and 45 days in order to evaluation of GFAP protein expression. control group; (B) the graph is representing the relative expression of GFAP protein. In the control group, GFAP protein synthesis is seen in the cortical cell line (to a lesser extent), while in the experimental groups of 15, 28, and 45 days, the synthesis of this protein increased over time. In the group receiving aflatoxin G1 for 45 days, protein synthesis increased significantly. ***P* < 0.01 and ****P* < 0.001 indicate significant differences compared to the control group. GFAP, Glial fibrillary acidic protein.

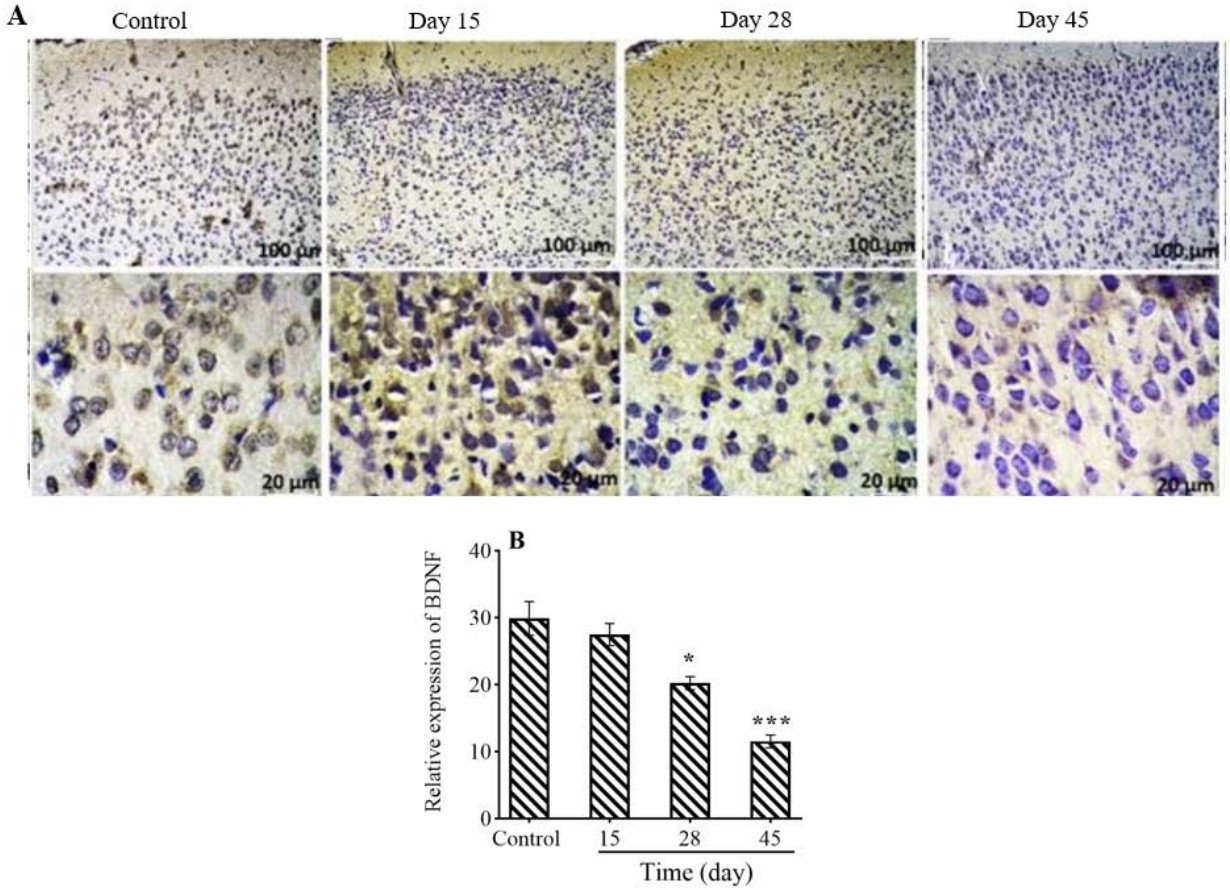

Fig. 6. (A) Immunohistochemical staining of the cerebral cortex of the rats receiving aflatoxin G1 at 2 mg/kg for 15, 28, and 45 days in order to evaluation of BDNF protein expression. control group; (B) the graph is representing the relative expression of BDNF protein. In the control group, the synthesis of BDNF protein is seen in the cortical cell line (to a large extent), while over time, the synthesis of this protein has decreased. In the 45-day group receiving aflatoxin G1, the rate of protein synthesis decreased significantly compared to the control group. *P < 0.05 and ***P < 0.001 indicate significant differences compared to the control group. BDNF, Brain-derived neurotrophic factors.

BDNF expression decreased significantly in the 15-, 28-, and 45-day groups receiving AFG-1 compared to the control group (Fig. 4B). The lowest expression of this gene was observed on day 45 after receiving AFG-1. An immunohistochemical study of BDNF protein showed that over time, the synthesis of this protein in the groups receiving AFG1 decreased significantly compared to the control group. The lowest synthesis of this protein was observed on day 45 following AFG1 administration (Fig. 6).

DISCUSSION

Our results showed that the tissue level of TAC and SOD in the groups receiving AFG1 had a statistically significant decrease over time compared to the control group and the tissue level of MDA in the groups receiving AFG1 had a statistically significant increase over time compared to the control group. Oxidative stress and decreased antioxidant activity predispose individuals to neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's disease, multiple sclerosis, and other complications in aging (23).

Decreased number of EM66-containing neurons in the arch nucleus, hypothalamic dysfunction of involved neuropeptides, weight gain, several histopathological changes of cell degeneration, dilation of blood vessels, and significant reduction of the thickness of the frontal cortex and the pyramidal cell layer of the hippocampal CA1 due to AFB1 have been reported (9,24). Long-term exposure to AFB1 leads to several pathophysiological complications, including necrosis, vasodilation, astrogliosis, and subsequent impairment of nerve damage (8). AF reacts with cellular

compounds such as nucleic acids, proteins, and important enzymes in the cell cycle pathway, leading to cell apoptosis, impaired neuronal function, and reduced neurodegenerative nervous system function (25,26).

Various studies have shown that toxic agents cause oxidative stress by altering total antioxidant levels, SOD, and MDA levels (27,28). A study about the neuroprotective effects of quercetin against AFB1-poisoned mice showed toxin-induced oxidative stress (29). Also, the activity level of MDA, SOD, CAT, and plasma glutathione peroxidase in the brain after AFB1 exposure in female rats increased (30).

RT-PCR results of this study showed that the expression of GFAP, especially in the cortex of brain tissue was increased significantly in the groups that received AFG1, compared to the control group. Immunohistochemically results also showed that AF increased the synthesis of GFAP protein in the cerebral cortex over time in the groups receiving AFG1 compared with the control group.

Various studies have shown that the administration of various toxins increases the expression of GFAP, which leads to astrogliosis (31-33). Also, in neurodegenerative diseases, the expression of the GFAP increases. Following brain and spinal cord injury and stroke, GFAP and its degradation products are rapidly released into biological fluids (34,35). The results of a study also show that AFB1 causes *in-vitro* and *in-vivo* cytotoxic effects on human astrocytes and zebrafish (36).

Real-time PCR results showed that BDNF expression in the groups receiving AFG1 was significantly reduced compared to the control group. Immunohistochemically study of BDNF protein also showed that the synthesis of this protein was significantly reduced over time in the groups receiving AFG1 compared to the control group. Because BDNF plays an important role in synaptic plasticity, memory, and neurogenesis, reducing its expression causes disorders in neurogenesis and nervous system function.

The damage to brain tissue caused by AFG1 could be corroborated by the key role of the GFAP gene in the activation of astroglia (astrogliosis) following CNS injury and neuronal destruction. BDNF is a member of the neurotrophin family, which has been shown to promote neurogenesis and is regulated by cholinergic and dopaminergic inputs in the hippocampus (37). Decreased amount of BDNF has been observed in the serum of people with Alzheimer's disease as well as in oxidative stress (38,39). The study also investigated the role of AFB1 in suppressing cholinergic signals on hilar GABAergic interneurons and neurotrophic factor-tropomyosin receptor kinase B signaling from brain-derived granular cells (7).

AFB1 can cause an inflammatory response in human microglial cells which is potentially harmful or toxic to the human central nervous system homeostasis and may increase the susceptibility to neurodegenerative diseases (40). The histopathological results of this study showed that the AFG1 effect was time-dependent on cortical tissue in rats. It can cause cell necrosis and reduce the number of normal cells in the hippocampal region of CA1, dilate perivascular space (cerebral edema), shrink nerve cells and form space around neuroglia, diffuse gliosis, and single neuron necrosis in the cerebral cortex after 45 days. Increased GFAP expression by AFG1 in turn indicated that the brain tissue was damaged because this gene is produced by astrocytes at the site of injury. In addition, decreased BDNF expression as an important neurogenic factor for the growth and development of the central nervous system has been able to influence the process of exacerbation of damage and destruction of nerve tissue. Decreased levels of SOD and TAC in brain tissue and increased levels of MDA-induced oxidative stress, among other factors, provide the basis for neurodegenerative diseases in the future.

Acknowledgments

This work was financially supported by the Pharmaceutical Sciences Research Centers of Kermanshah University of Medical Sciences, Kermanshah, I.R. Iran through Grant No. 4010137.

Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

T. Zamir-Nasta developed the hypothesis and performed the literature search. T. Zamir-Nasta, A. Abbasi, S. Kakebaraie, A. Ahmadi, and M.Pazhouh contributed to the design and conceptualized the experiments described. T. Zamir-Nasta and A. Abbasi analyzed the data and wrote the manuscript. C. Jalili carried out a thorough analysis of the text. The final version of the manuscript was approved by all authors.

REFERENCES

1. Dhanasekaran D, Shanmugapriya S, Thajuddin N, Panneerselvam A. Aflatoxins and Aflatoxicosis in Human and Animals. In: Guevara-Gonzalez RG, editor. Aflatoxins - Biochemistry and Molecular Biology. IntechOpen; 2011. pp. 221-254. DOI: 10.5772/22717.
2. Shen H, Lv P, Xing X, Xing L, Yan X, Wang J, *et al*. Impairment of alveolar type-II cells involved in the toxicity of aflatoxin G1 in rat lung. Food Chem Toxicol. 2012;50(9):3222-3228. DOI: 10.1016/j.fct.2012.06.008.
3. Khan MR, Alothman ZA, Ghfar AA, Wabaidur SM. Analysis of aflatoxins in nonalcoholic beer using liquid-liquid extraction and ultraperformance LC-MS/MS. J Sep Sci. 2013;36(3):572-577. DOI: 10.1002/jssc.201200752.
4. Zhang X, Wang F, Wang J, Yan X, Huang X, Xie T, *et al*. Experimental lung carcinoma induced by fungi and mycotoxins--a review. Beijing Da Xue Xue Bao Yi Xue Ban. 2003;35(1):4-6. PMID: 12920799.
5. Zhang Z, Yang X, Wang Y, Wang X, Lu H, Zhang X, *et al*. Cytochrome P450 $_2A_{13}$ is an efficient enzyme in the metabolic activation of aflatoxin G1 in human bronchial epithelial cells. Arch Toxicol. 2013;87(9):1697-1707. DOI: 10.1007/s00204-013-1108-3.
6. Makhlouf MMM. Histological and ultrastructural study of aflatoxinB1 induced neurotoxicity in sciatic nerve of adult male Albino rats. Ultrastruct Pathol. 2020;44(1):52-60. DOI: 10.1080/01913123.2019.1709933.
7. Tanaka T, Mizukami S, Hasegawa-Baba Y, Onda N, Sugita-Konishi Y, Yoshida T, *et al*. Developmental exposure of aflatoxin B1 reversibly affects hippocampal neurogenesis targeting late-stage neural progenitor cells through suppression of cholinergic signaling in rats. Toxicology. 2015;336:59-69. DOI: 10.1016/j.tox.2015.08.001.
8. Alsayyah A, ElMazoudy R, Al-Namshan M, Al-Jafary M, Alaqeel N. Chronic neurodegeneration by aflatoxin B1 depends on alterations of brain enzyme activity and immunoexpression of astrocyte in male rats. Ecotoxicol Environ Saf. 2019;182:109407,1-9. DOI: 10.1016/j.ecoenv.2019.109407.
9. Bahey NG, Abd Elaziz HO, Gadalla KKES. Toxic effect of aflatoxin B1 and the role of recovery on the rat cerebral cortex and hippocampus. Tissue Cell. 2015;47(6):559-566. DOI; 10.1016/j.tice.2015.09.001.
10. Dohnal V, Wu Q, Kuča K. Metabolism of aflatoxins: key enzymes and interindividual as well as interspecies differences. Arch Toxicol. 2014;88(9):1635-1644. DOI: 10.1007/s00204-014-1312-9.
11. Qureshi H, Hamid SS, Ali SS, Anwar J, Siddiqui AA, Khan NA. Cytotoxic effects of aflatoxin B1 on human brain microvascular endothelial cells of the blood-brain barrier. Med Mycol. 2015;53(4):409-416. DOI: 10.1093/mmy/myv010.
12. Tapia-Arancibia L, Rage F, Givalois L, Arancibia S. Physiology of BDNF: focus on hypothalamic function. Front Neuroendocrinol. 2004;25(2):77-107. DOI: 10.1016/j.yfrne.2004.04.001.
13. Ogłodek EA, Just MJ, Szromek AR, Araszkiewicz A. Melatonin and neurotrophins NT-3, BDNF, NGF in patients with varying levels of depression severity. Pharmacol Rep. 2016;68(5):945-951. DOI: 10.1016/j.pharep.2016.04.003.
14. Radiske A, Rossato JI, Köhler CA, Gonzalez MC, Medina JH, Cammarota M. Requirement for BDNF in the reconsolidation of fear extinction. J Neurosci. 2015;35(16):6570-6574. DOI: 10.1523/JNEUROSCI.4093-14.2015.
15. Vafaei AA, Jezek K, Bures J, Fenton AA, Rashidy-Pour A. Post-training reversible inactivation of the rat's basolateral amygdala interferes with hippocampus-dependent place avoidance memory in a time-dependent manner. Neurobiol Learn Mem. 2007;88(1):87-93. DOI: 10.1016/j.nlm.2007.02.004.
16. Moeton M, Stassen OM, Sluijs JA, van der Meer VW, Kluivers LJ, van Hoorn H, *et al*. GFAP isoforms control intermediate filament network dynamics, cell morphology, and focal adhesions. Cell Mol Life Sci. 2016;73(21):4101-4120. DOI: 10.1007/s00018-016-2239-5.
17. Batarseh YS, Mohamed LA, Al Rihani SB, Mousa YM, Siddique AB, El Sayed KA, *et al*. Oleocanthal ameliorates amyloid-β oligomers' toxicity on astrocytes and neuronal cells: *in vitro* studies. Neuroscience. 2017;352:204-215. DOI .10.1016/j.neuroscience.2017.03.059.
18. Barreto RA, Sousa CS, Silva VDA, Silva AR, Veloso ES, Cunha SD, *et al*. Monocrotaline pyrrol is cytotoxic and alters the patterns of GFAP expression on astrocyte primary cultures. Toxicol In Vitro. 2008;22(5):1191-1197. DOI: 10.1016/j.tiv.2008.03.013.
19. Zamir-Nasta T, Pazhouhi M, Ghanbari A, Abdolmaleki A, Jalili C. Expression of cyclin D1, p21, and estrogen receptor alpha in aflatoxin G1-induced disturbance in testicular tissue of albino mice. Res Pharm Sci. 2021;16(2):182-192. DOI: 10.4103/1735-5362.310525.
20. Deng ZJ, Zhao JF, Huang F, Sun GL, Wei G, Li L, *et al*. Protective effect of procyanidin B2 on acute liver injury induced by aflatoxin B1 in rats. Biomed Environ Sci. 2020;33(4):238-247. DOI: 0.3967/bes2020.033.

21. Ashraf B, Ghazy D, Shamel M. Effects of aflatoxin B1 on the submandibular salivary gland of albino rats and possible therapeutic potential of *Rosmarinus officinalis*: a light and electron microscopic study. F1000Res. 2020;9:752. DOI: 10.12688/f1000research.25196.1.
22. Jalili C, Salahshoor MR, Pourmotabbed A, Moradi S, Roshankhah SH, Darehdori AS, *et al*. The effects of aqueous extract of *Boswellia serrata* on hippocampal region CA1 and learning deficit in kindled rats. Res Pharm Sci. 2014;9(5):351-358. PMID: 25657807.
23. Stephenson J, Nutma E, P der Valk, S Amor, Inflammation in CNS neurodegenerative diseases. Immunology. 2018;154(2):204-219. DOI: 10.1111/imm.12922.
24. Trebak F, Alaoui A, Alexandre D, El Ouezzani S, Anouar Y, Chartrel N, *et al*. Impact of aflatoxin B1 on hypothalamic neuropeptides regulating feeding behavior. Neurotoxicology. 2015;49:165-173. DOI: 10.1016/j.neuro.2015.06.008.
25. Amici M, Cecarini V, Pettinari A, Bonfili L, Angeletti M, Barocci S, *et al*. Binding of aflatoxins to the 20S proteasome: effects on enzyme functionality and implications for oxidative stress and apoptosis. Biol Chem. 2007;388(1):107-117. DOI: 10.1515/BC.2007.012.
26. Benkerroum N. Chronic and acute toxicities of aflatoxins: mechanisms of action. Int J Environ Res Public Health. 2020;17(2):423,1-28. DOI: 10.3390/ijerph17020423.
27. Al-Rubaei ZMM, Mohammad TU, Ali LK. Effects of local curcumin on oxidative stress and total antioxidant capacity *in vivo* study. Pak J Biol Sci. 2014;17(12):1237-1241. DOI: 10.3923/pjbs.2014.1237.1241.
28. Souto NS, Braga ACM, de Freitas ML, Fighera RM, Royes LFF, Oliveira MS, *et al*. Aflatoxin B1 reduces non-enzymatic antioxidant defenses and increases protein kinase C activation in the cerebral cortex of young rats. Nutr Neurosci. 2018;21(4):268-275. DOI: 10.1080/1028415X.2017.1278837.
29. Gugliandolo E, Peritore AF, D'Amico R, Licata P, Crupi R. Evaluation of neuroprotective effects of quercetin against aflatoxin B1-intoxicated mice. Animals (Basel). 2020;10(5):898,1-13. DOI: 10.3390/ani10050898.
30. Karabacak M, Eraslan G, Kanbur M, Sarıca ZS. Effects of *Tarantula cubensis* D6 on aflatoxin-induced injury in biochemical parameters in rats. Homeopathy. 2015;104(3):205-210. DOI: 10.1016/j.homp.2015.02.005.
31. Boccoli J, Loidl CF, Lopez-Costa JJ, Creydt VP, Ibarra C, Goldstein J. Intracerebroventricular administration of Shiga toxin type 2 altered the expression levels of neuronal nitric oxide synthase and glial fibrillary acidic protein in rat brains. Brain Res. 2008;1230:320-333. DOI: 10.1016/j.brainres.2008.07.052.
32. Vazi E, Holanda F, Santos N, Cardoso C, Martins M, Bondan E. Short-term systemic methotrexate administration in rats induces astrogliosis and microgliosis. Res Vet Sci. 2021;138:39-48. DOI: 10.1016/j.rvsc.2021.05.020.
33. Freyssin A, Fauconneau B, Chassaing D, Rioux Bilan A, Page G. Chronic intraperitoneal injection of polyethylene glycol 200 in mice induces hippocampal neuroinflammation. Drug Chem Toxicol. 2022;45(5):1995-2002. DOI: 10.1080/01480545.2021.1894738.
34. Ferrer I. Diversity of astroglial responses across human neurodegenerative disorders and brain aging. Brain Pathol. 2017;27(5):645-674. DOI: 10.1111/bpa.12538.
35. Yang Z, Wang KKW. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci. 2015;38(6):364-374. DOI: 10.1016/j.tins.2015.04.003.
36. Park S, Lee JY, You S, Song G, Lim W. Neurotoxic effects of aflatoxin B1 on human astrocytes *in vitro* and on glial cell development in zebrafish *in vivo*. J Hazard Mater. 2020;386:121639,1-40. DOI: 10.1016/j.jhazmat.2019.121639.
37. Liu PZ, Nusslock R. Exercise-mediated neurogenesis in the hippocampus *via* BDNF. Front Neurosci. 2018;12:52,1-6. DOI: 10.3389/fnins.2018.00052.
38. Siang Ng TK, Hui Ho CS, Tam WWS, Kua EH, Ho RCM. Decreased serum brain-derived neurotrophic factor (BDNF) levels in patients with Alzheimer's disease (AD): a systematic review and meta-analysis. Int J Mol Sci. 2019;20(2):257,1-26. DOI: 10.3390/ijms20020257.
39. Wei C, Sun Y, Chen N, Chen S, Xiu M, Zhang X. Interaction of oxidative stress and BDNF on executive dysfunction in patients with chronic schizophrenia. Psychoneuroendocrinology. 2020;111:104473,1-7. DOI: 10.1016/j.psyneuen.2019.104473.
40. Mehrzad J, Hosseinkhani S, Malvandi AM. Human microglial cells undergo proapoptotic induction and inflammatory activation upon *in vitro* exposure to a naturally occurring level of aflatoxin B1. Neuroimmunomodulation. 2018;25(3):176-183. DOI: 10.1159/000493528.