Received: 6 August 2020 | Revised: 7 October 2020 | Accepted: 26 November 2020

DOI: 10.1002/jbt.22684

JOURNAL OF BIOCHEMICAL AND MOLECULAR TOXICOLOGY WILEY

Neuroprotective role of gallic acid in aflatoxin B_1-induced behavioral abnormalities in rats

Isaac A. Adedara[1] | Solomon E. Owumi[2] | Adegboyega K. Oyelere[3] | Ebenezer O. Farombi[1]

[1]Department of Biochemistry, Drug Metabolism and Toxicology Research Laboratories, College of Medicine, University of Ibadan, Ibadan, Nigeria

[2]Department of Biochemistry, Cancer Research and Molecular Biology Laboratory, College of Medicine, University of Ibadan, Ibadan, Nigeria

[3]School of Biochemistry and Chemistry, Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia, USA

Correspondence
Isaac A. Adedara, Department of Biochemistry, Drug Metabolism and Toxicology Research Laboratories, College of Medicine, University of Ibadan, Ibadan, Nigeria.
Email: dedac2001@yahoo.co.uk

Abstract

The neurotoxic impact of dietary exposure to aflatoxin B_1 (AFB_1) is documented in experimental and epidemiological studies. Gallic acid (GA) is a triphenolic phytochemical with potent anticancer, anti-inflammatory, and antioxidant activities. There is a knowledge gap on the influence of GA on AFB_1-induced neurotoxicity. This study probed the influence of GA on neurobehavioral and biochemical abnormalities in rats orally treated with AFB_1 per se (75 μg/kg body weight) or administered together with GA (20 and 40 mg/kg) for 28 uninterrupted days. Behavioral endpoints obtained with video-tracking software demonstrated significant ($p < .05$) abatement of AFB_1-induced anxiogenic-like behaviors (increased freezing, urination, and fecal bolus discharge), motor and locomotor inadequacies, namely increased negative geotaxis and diminished grip strength, absolute turn angle, total time mobile, body rotation, maximum speed, and total distance traveled by GA. The improvement of exploratory behavior in animals that received both AFB_1 and GA was confirmed by track plots and heat maps appraisal. Abatement of AFB_1-induced decreases in acetylcholinesterase activity, antioxidant status and glutathione level by GA was accompanied by a marked reduction in oxidative stress markers in the cerebellum and cerebrum of rats. Additionally, GA treatment abrogated AFB_1-mediated decrease in interleukin-10 and elevation of inflammatory indices, namely tumor necrosis factor-α, myeloperoxidase activity, interleukin-1β, and nitric oxide. Further, GA treatment curtailed caspase-3 activation and histological injuries in the cerebral and cerebellar tissues. In conclusion, abatement of AFB_1-induced neurobehavioral abnormalities by GA involves anti-inflammatory, antioxidant, and antiapoptotic mechanisms in rats.

KEYWORDS

aflatoxin B_1, apoptosis, gallic acid, neurotoxicity, oxido-inflammation

1 | INTRODUCTION

Aflatoxins are naturally occurring mycotoxins with foremost deleterious consequences on animal and human health. Aflatoxin B_1 (AFB_1), which is produced by *Aspergillus flavus* and *Aspergillus parasiticus*, is the most predominant and poisonous form of aflatoxins.[1] The presence of AFB_1 in foods and feedstuffs has been detected in contaminated peanuts, corn, wheat, cotton seeds, fruit juices, milk, and several agricultural produces.[2,3] Dietary exposure to AFB_1 is reported to be more in the tropical than temperate regions owing to favorable

humidity and hot climates, which are essential for fungi's growth. However, the prevalent harmful impact of AFB_1-contamination of food products in the world is associated with the extensive transaction of agricultural foodstuffs.[4] Acute exposure of humans to aflatoxins is often characterized by abdominal pain, convulsions, vomiting, coma, and death with cerebral edema.[5,6] The toxic effects of AFB_1 in exposed organisms have been documented to involve metabolic activation by cytochrome P-450 (CYP450)-dependent monooxygenase to its reactive AFB1-8,9-epoxide, which subsequently interferes with cellular macromolecules.[7,8]

Dietary exposure to AFB_1 is a worldwide health burden because AFB_1 has been implicated in hepatotoxicity, carcinogenesis, immunotoxicity, reproductive dysfunction, and neurotoxicity.[5,9] An epidemiological report revealed that AFB_1 was detected in autopsy brain samples of children in Nigeria.[10] Moreover, AFB_1 reportedly enters the brain via destruction of the blood-brain barrier's integrity and consequently induces cognitive deficits.[11] Previous neurotoxicity data from animal models showed that exposure to AFB_1 significantly impaired locomotor function and disrupted neural development in zebrafish embryos and larvae.[12] Moreover, prenatal exposure to AFB_1 markedly delayed learning ability, the development of reflex response with attendant locomotor deficits in rat offspring.[13] Neurobiochemical modifications associated with AFB_1 exposure in animals include inhibition of enzymes involved in cholinergic neurotransmission, oxidative phosphorylation, and antioxidant defense,[14,15] as well as induction of oxidative stress.[16] Histopathological lesions associated with AFB_1 exposure include diminution of nerve fibers and neuronal degeneration in the frontal cortex and hippocampus.[17] Thus, in view of the various harmful impact of AFB_1 on human and animal health, research into the possible antidotes for toxicities due to AFB_1 exposure is warranted.

Gallic acid (GA), otherwise known as 3,4,5-trihydroxybenzoic acid, is an important low molecular triphenolic phytochemical commonly found in numerous vendible beverages, specifically green tea, coffee, red wine, and pomegranate juice.[18] Beneficial health effects of GA have been related to its anticancer, antimicrobial, anti-inflammatory, and antioxidant activities.[19,20] Industrially, GA is an essential food additive for the prevention of rancidity of fats and oils.[21] Earlier investigations in rodents showed that GA protected against neurotoxicity associated with glutamate,[22] cobalt chloride,[23] arsenic,[24] and aluminum chloride.[25] Besides, GA effectively diminished neuroinflammation induced by lipopolysaccharides by attenuating protein aggregation and necroptosis in rats.[20] GA reportedly protected against ketamine-induced memory impairments and lesions in the brain of rats,[26] rescued cognitive failure in APP/PS1 double transgenic mouse via interference with Aβ(1-42) aggregation,[27] and effectively inhibited aflatoxin production in *Aspergillus flavus*.[28] Recent studies from our laboratories demonstrated the ameliorative influence of GA on AFB_1-mediated hepatorenal and reproductive damage in rats.[29,30] However, scientific evidence on the role of GA in the neurotoxicity linked to AFB_1 exposure is lacking. We hypothesized that owing to the widely reported intrinsic beneficial biological activities of this ubiquitous phytochemical, GA may play a significant role in ameliorating AFB_1-induced neurotoxicity.

The current study aimed at examining, for the first time, the influence of GA on AFB_1-associated neurotoxicity in rats by assessing the locomotor and exploratory characteristics using video-tracking software and an established neurobehavioral technique in an open field.[31] Thereafter, biochemical assays comprising activity acetylcholinesterase (AChE), inflammation, antioxidant enzyme activities, indices of oxidative stress, as well as histomorphometrical and histological examination of the cerebellum and cerebrum of rats were done.

2 | MATERIALS AND METHODS

2.1 | Chemicals and assay kits

GA (≥95%) and AFB_1 (≥98%) were purchased from Sigma-Aldrich Chemical Co. Evaluation of interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and caspase-3 were done using enzyme-linked immunosorbent assay (ELISA) kits procured from Elabscience Biotechnology Company.

2.2 | Care of experimental rats

Fifty male rats (Wistar strain, 8 weeks old, 156 ± 5 g) used for the current research were gotten from the Faculty of Veterinary Medicine, University of Ibadan. The rats were accommodated in standard cages sited in an aired animal room with 12 h light:12 h dark photocycle. The rats were given drinking water and rat food ad libitum and acclimatized for a week before administration of test compounds. An ample amount of wood shavings was supplied as bedding. The care of animals and treatment approach were executed after institutional approval and in conformity with the ratified rules of the University of Ibadan Ethical Committee and the U.S. National Institute of Health.

2.3 | Research design

Five groups of 10 rats each were treated for 28 successive days as follows.

Control: Rats administered 2 ml/kg body weight (bw) of corn oil alone.

GA alone: Rats administered 40 mg/kg bw of GA alone.

AFB1 alone: Rats administered 75 μg/kg bw of AFB1 alone.

AFB1 + GA1: Rats administered 75 μg/kg bw of AFB1 and 20 mg/kg of GA.

AFB1 + GA2: Rats administered 75 μg/kg bw of AFB1 and 40 mg/kg of GA.

AFB1 and GA doses administered in the current investigation were chosen from previously published articles.[29,30]

2.4 | Evaluation of behavior and exploratory accomplishments

The behavior and exploratory accomplishments of rats in all the groups were monitored on Day 29 in a novel wooden box (56 cm length × 56 cm width × 20 cm height) in line with the established technique.[31] Briefly, each rat was put in the box and allowed to freely roam for 8 min, while the behavior was captured by a webcam (DNE webcam) directly sited overhead the novel box. The videotapes were analyzed by video-tracking software (ANY-maze, Stoelting Co). The box was thoroughly cleaned with 70% ethanol and cotton wool after each trial. Automatically generated locomotor parameters were total time mobile, maximum speed, total distance traveled, path efficiency, absolute turn angle, body rotation, and total freezing time, whereas track plots and heat maps represent exploratory activities of the rats in the novel apparatus. In accordance with the established protocols, a negative geotaxis test was done to appraise the motor fitness,[32] whereas a forelimb grip test was performed to evaluate the strength of the limbs of animals.[33,34] Further, fear or anxiety-related behavior was assessed by recording the frequency of urination and fecal pellets release according to the established protocol.[34,35]

2.5 | Assay of AChE activity and oxido-inflammatory stress indices

The rats were weighed and killed using light ether anesthesia following behavioral assessment. The brain was carefully excised and separated into cerebrum and cerebellum before homogenization in ten volumes of 50 mM Tris–HCl buffer (pH 7.4). Subsequently, the homogenate was centrifuged (12,000*g* for 15 min at 4°C) and the supernatants of the cerebrum and cerebellum were used for biochemical assays. Protein concentration was assessed using the standard protocol.[36] AChE activity was analyzed in accordance with Ellman et al.[37] Activities of antioxidant enzymes, namely catalase (CAT) was assayed in accordance with Claiborne,[38] superoxide dismutase (SOD) in accordance with Misra and Fridovich,[39] glutathione peroxidase (GPx) in accordance with Rotruck et al.,[40] and glutathione-*S*-transferase (GST) in accordance with Habig et al.[41] The level of glutathione (GSH) was assayed, as described by Jollow et al.[42]

Oxidative stress parameters, precisely the levels of reactive oxygen and nitrogen species (RONS) and lipid peroxidation (LPO), were evaluated in accordance with Farombi et al.[43] and Adedara et al.[44] respectively. Inflammatory indices, namely nitric oxide (NO) level and myeloperoxidase (MPO) activity, were appraised using standard procedures by Green et al.[45] and Granell et al.,[46] respectively. Besides, TNF-α, IL-1β, and IL-10 levels, as well as caspase-3 activity were analyzed using ELISA Kits as itemized in the manufacturer's guide. Activities of CAT and SOD were analyzed using 752S UV-VIS Spectrophotometer, whereas all other biochemical estimations were performed using a SpectraMax plate reader (Molecular Devices).

2.6 | Histological and histomorphometrical evaluation

Histological and histomorphometrical assessment of the cerebellum and cerebrum was executed using 5 μm sections with hematoxylin and eosin stain in accordance with the established practice.[47] The quantification of Purkinje and cortical neurons was done by counting both the viable and degenerated neurons using a graticule and a microscope at varied magnifications. A minimum of 10 observations was charted in each slide and the average values were computed for each group. Viable neurons were identified by the presence of distinct nucleoli, dispersed chromatin, and absence of cell death features, specifically karyolysis and pyknosis, at high power by pathologists. The quantity of degenerated Purkinje and cortical neurons was presented as percentages.

2.7 | Statistical analyses

One-way analysis of variance and Bonferroni's post hoc test using GraphPad Prism 5 software (Version 4; GraphPad Software) were used to analyze the results of the present study. Values of $p < .05$ were considered significant.

3 | RESULTS

3.1 | GA abrogated AFB_1-induced anxiogenic-like behavior and motor activity decline in rats

Anxiogenic-like behavior and motor activity of rats administered AFB_1 per se or cotreated with GA are presented in Figure 1. AFB_1 alone-exposed rats displayed more anxiogenic-like behaviors evidenced by increased freezing time and frequency of urination and fecal bolus discharge than control rats. Moreover, AFB_1 alone-exposed rats displayed less motor activity evidenced by significant ($p < .05$) decrease in grip strength and increased negative geotaxis compared with control. Effects of GA alone on anxiogenic-like behavior and motor activity were comparable with control. Rats cotreated with AFB_1 and GA at 20 and 40 mg/kg evidently showed reduced freezing time and frequency of urination and fecal bolus discharge, negative geotaxis with concomitant improvement in the grip strength compared with AFB_1 alone group.

3.2 | GA abated AFB_1-induced impairment of exploratory and locomotor actions in rats

Figures 2 and 3 show the influence of GA on exploratory and locomotor actions of AFB_1-treated rats. Exposure to AFB_1 alone significantly impaired locomotor actions of the rats as evidenced

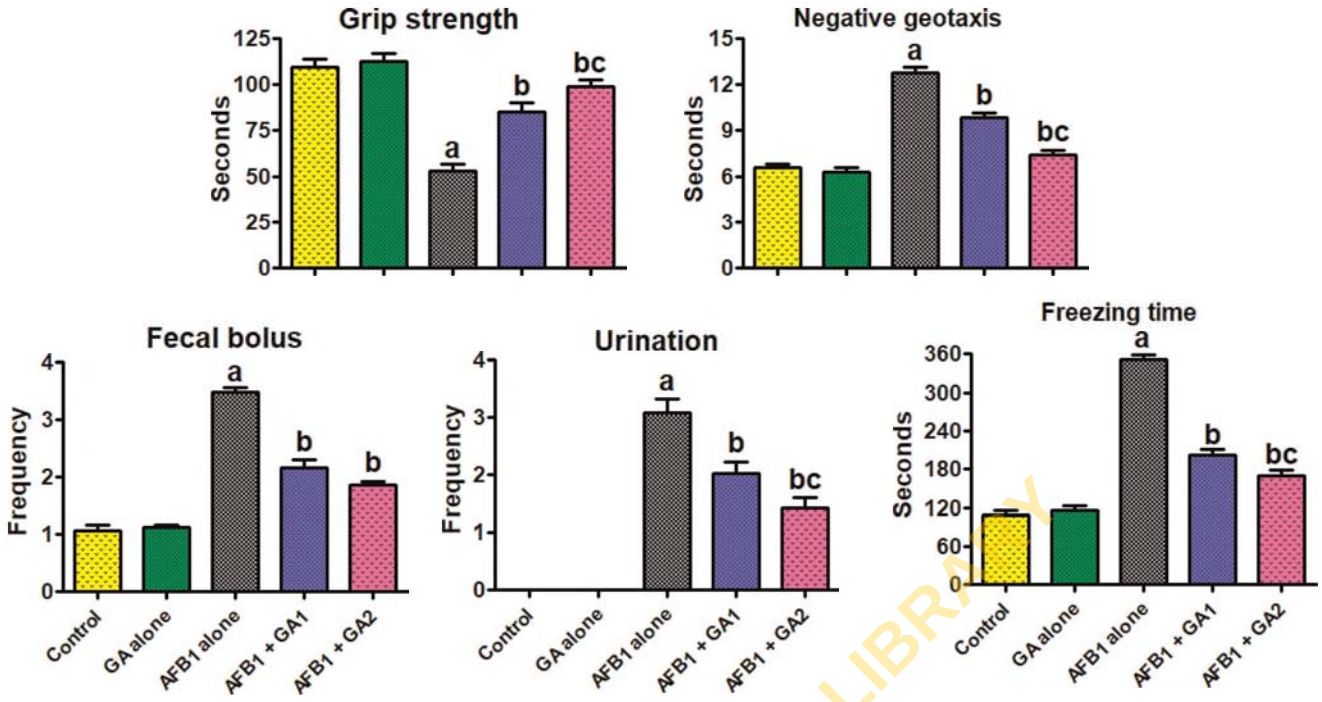

FIGURE 1 Impact of GA on anxiogenic-like behavior and motor activity in AFB_1-treated rats. GA1 and GA2 denote Gallic acid at 20 and 40 mg/kg, respectively; AFB_1 denotes aflatoxin B_1 at 75 μg/kg. Data are presented as mean ± *SD* for 10 rats per group. AFB_1, aflatoxin B_1; GA, gallic acid. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from AFB_1 alone ($p < .05$). [c]Values differ significantly from AFB_1 + GA1 ($p < .05$)

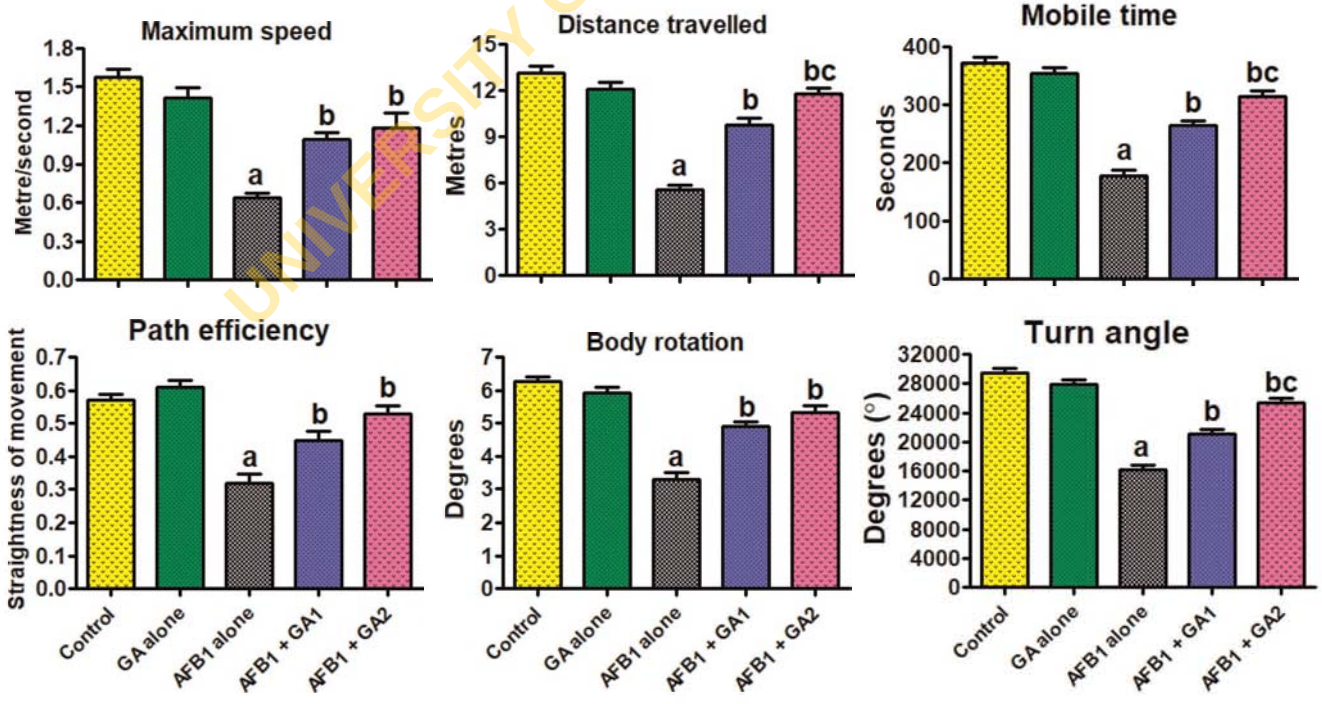

FIGURE 2 Impact of GA on locomotor activity of AFB_1-treated rats. GA1 and GA2 denote gallic acid at 20 and 40 mg/kg, respectively; AFB_1 denotes aflatoxin B_1 at 75 μg/kg. Data are presented as mean ± *SD* for 10 rats per group. AFB_1, aflatoxin B_1; GA, gallic acid. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from AFB_1 alone ($p < .05$). [c]Values differ significantly from AFB_1 + GA1 ($p < .05$)

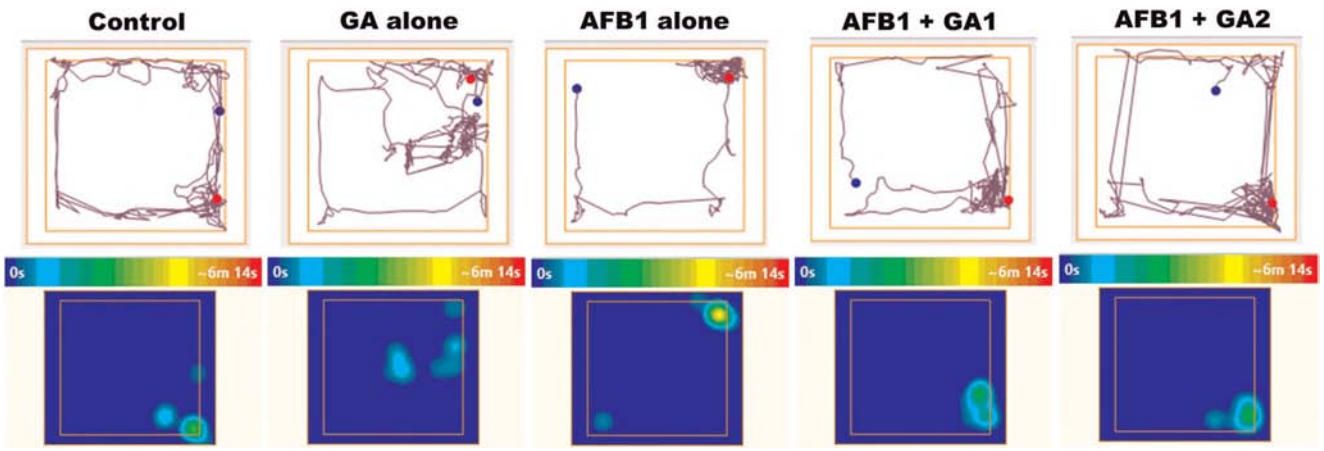

FIGURE 3 Impact of GA on exploratory behavior of AFB_1-treated rats. GA1 and GA2 denote gallic acid at 20 and 40 mg/kg, respectively; AFB_1 denotes aflatoxin B_1 at 75 μg/kg. The data were analyzed using video-tracking software (ANY-maze; Stoelting Co). AFB_1, aflatoxin B_1; GA, gallic acid

by decreased time mobile, body rotation, maximum speed, absolute turn angle, and total distance traveled. Further, exposure to AFB_1 alone impaired exploratory competency as demonstrated by reduced track plot density and higher heat map intensity in the novel apparatus. Effects of GA alone on locomotor and exploratory actions were comparable with control. On the other hand, rats cotreated with AFB_1 and GA at 20 and 40 mg/kg demonstrated enhanced locomotor and exploratory actions compared with AFB_1 alone group.

3.3 | GA modulated AFB_1-induced decrease in antioxidant status and AChE activity in rats

Figures 4 and 5 portray the influence of GA treatment on AChE activity and antioxidant status in AFB_1-treated rats. Compared with control, AFB_1 treatment alone significantly diminished cerebral and cerebellar AChE activity, a biomarker for nervous system function, in the rats. Moreover, the level of GSH and activities of CAT, SOD, GST, and GPx in the cerebellum and cerebrum of rats treated with AFB_1

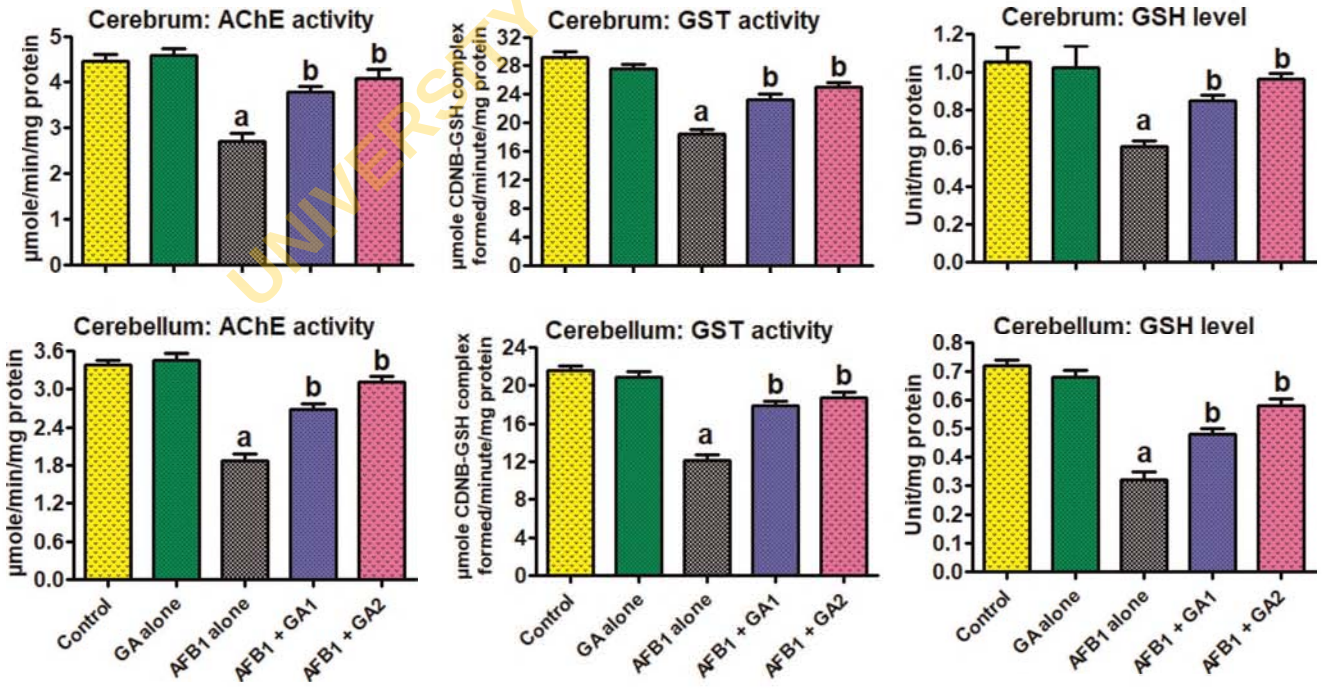

FIGURE 4 Impact of GA on AChE and GST activities as well as GSH level in cerebrum and cerebellum of AFB_1-treated rats. GA1 and GA2 denote Gallic acid at 20 and 40 mg/kg, respectively; AFB_1 denotes aflatoxin B_1 at 75 μg/kg. Data are presented as mean ± *SD* for 10 rats per group. AChE, acetylcholinesterase; AFB_1, aflatoxin B_1; GA, gallic acid; GSH, glutathione; GST, glutathione-*S*-transferase. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from AFB_1 alone ($p < .05$). [c]Values differ significantly from AFB_1 + GA1 ($p < .05$)

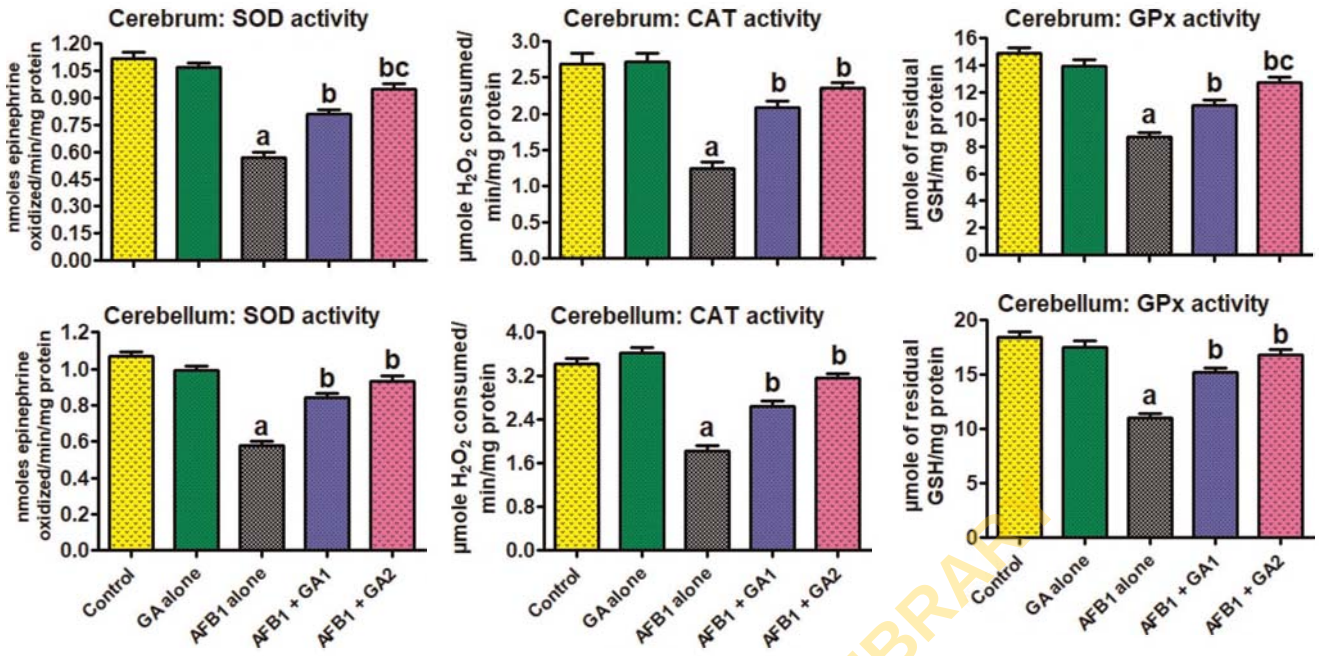

FIGURE 5 Impact of GA on SOD, CAT, and GPx activities in the cerebrum and cerebellum of AFB_1-treated rats. GA1 and GA2 denote gallic acid at 20 and 40 mg/kg, respectively; AFB_1 denotes aflatoxin B_1 at 75 µg/kg. Data are presented as mean ± *SD* for 10 rats per group. AFB_1, aflatoxin B_1; CAT, catalase; GA, gallic acid; GPx, glutathione peroxidase; SOD, superoxide dismutase. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from AFB_1 alone ($p < .05$). [c]Values differ significantly from AFB_1 + GA1 ($p < .05$)

alone were significantly decreased compared with the control. The impact of GA alone on AChE activity and antioxidant status were comparable to control. Rats cotreated with AFB_1 and GA at 20 and 40 mg/kg exhibited marked improvement in nervous system function and antioxidant status as evidenced by increased AChE and antioxidant enzyme activities alongside GSH level in the cerebellar and cerebral tissues.

3.4 | GA abated AFB_1-induced increase in oxido-inflammatory stress and caspase-3 activity

Biomarkers of inflammation, oxidative stress, and apoptosis in the cerebellum and cerebrum of rats treated with AFB_1 alone or cotreated with GA are shown in Figures 6–8. Treatment with AFB_1 alone caused a significant increase in biomarkers of oxidative stress, namely RONS and LPO levels, alongside inflammatory indices, specifically NO, TNF-α, and IL-1β levels and MPO activity compared with control. Moreover, the concentration of anti-inflammatory cytokine IL-10 in the cerebrum and cerebellum of rats treated with AFB_1 alone was significantly reduced compared with control. The effects of GA alone on oxido-inflammatory stress and caspase-3 activity were not different from control. The cotreatment with AFB_1 and GA at 20 and 40 mg/kg increased IL-10 concentration but diminished biomarkers of inflammation and oxidative stress in the cerebrum and cerebellum of the treated rats compared with AFB_1 alone group. Further, rats exposed to AFB_1 alone showed a significant increase in cerebral and cerebellar caspase-3 activity, whereas its activity was markedly decreased upon cotreatment with GA at 20 and 40 mg/kg when compared with AFB_1 alone group.

3.5 | GA abrogated histomorphometrical and histological modifications in AFB_1-exposed rats

Figures 9 and 10 are the representative photomicrographs of the cerebellum and cerebrum of rats exposed to AFB_1 alone or coadministered with GA. The histological appearance of the cerebellum and cerebrum from control and GA alone groups is similar to normal neurons. However, AFB_1 alone-exposed rats exhibited degenerated neurons with mild vacuolation of the neuropil, onset of pyknosis, and marked congestion in the cerebral cortex, whereas degenerated neurons in the Purkinje cell layer of the cerebellum was characterized with reduced nuclear material. The cerebral and cerebellar injuries due to AFB_1 intoxication were considerably ameliorated upon cotreatment with GA at 20 and 40 mg/kg.

4 | DISCUSSION

The current study demonstrated extrahepatic menace of AFB_1, which may cause neurobehavioral deficits in exposed populaces and that GA effectively abated AFB_1-induced neurotoxicity in exposed rats. Specifically, exposure to AFB_1alone triggered motor debility characterized by a decrease in body rotation, grip strength, and turn angle, which are key motor agility features

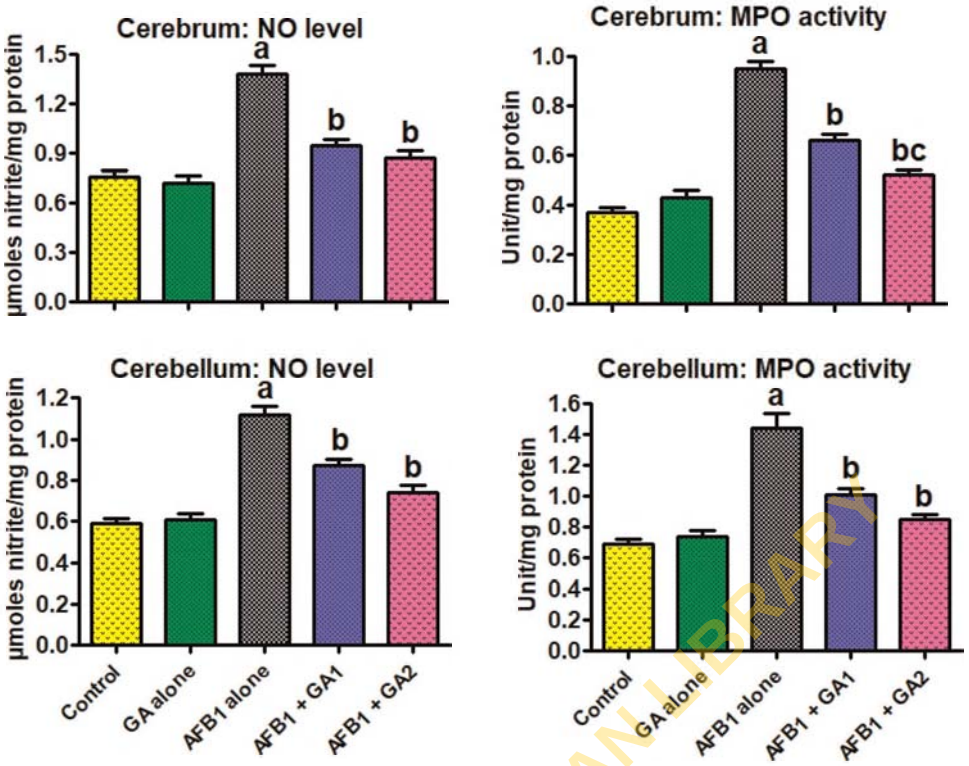

FIGURE 6 Impact of GA on MPO activity and NO level in cerebrum and cerebellum of AFB_1-treated rats. GA1 and GA2 denote gallic acid at 20 and 40 mg/kg, respectively; AFB_1 denotes aflatoxin B_1 at 75 µg/kg. Data are presented as mean ± *SD* for 10 rats per group. AFB_1, aflatoxin B_1; GA, gallic acid; MPO, myeloperoxidase; NO, nitric oxide. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from AFB_1 alone ($p < .05$). [c]Values differ significantly from AFB_1 + GA1 ($p < .05$)

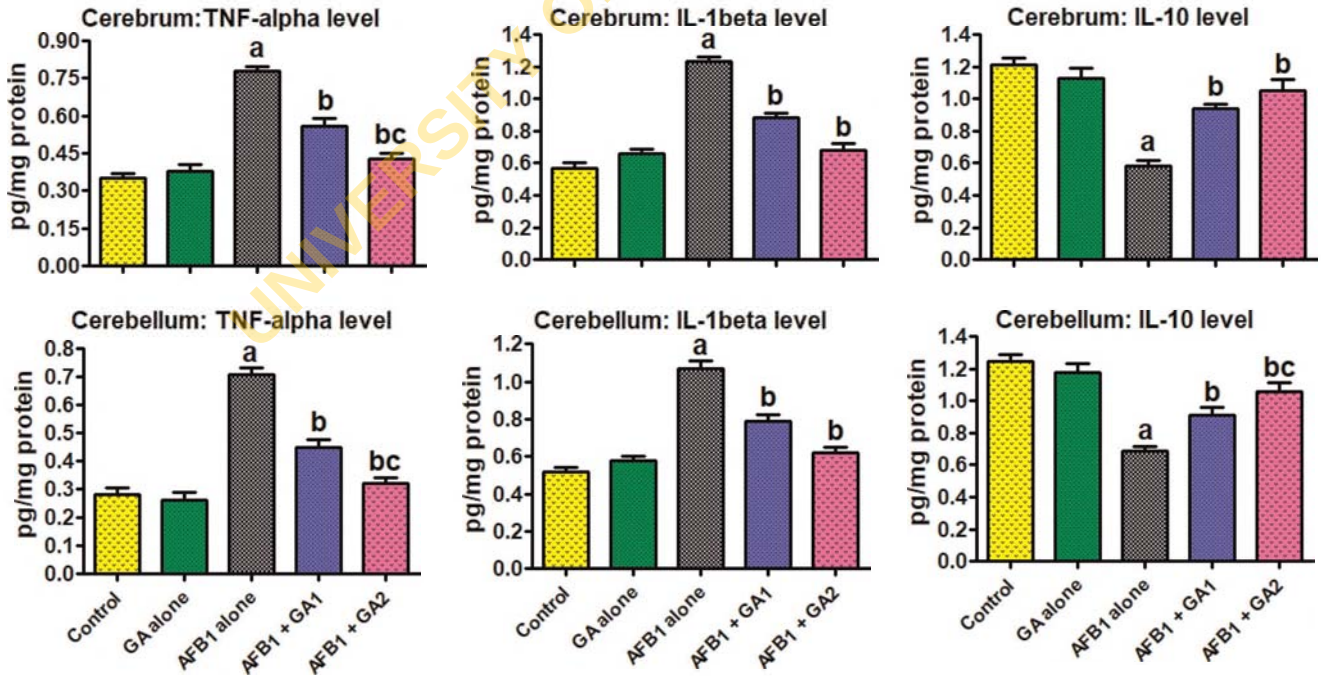

FIGURE 7 Impact of GA on TNF-α, IL-1β, and IL-10 levels in cerebrum and cerebellum of AFB_1-treated rats. GA1 and GA2 denote Gallic acid at 20 and 40 mg/kg, respectively; AFB_1 denotes aflatoxin B_1 at 75 µg/kg. Data are presented as mean ± *SD* for 10 rats per group. GA, gallic acid; AFB_1, aflatoxin B_1; IL-10, interleukin-10; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from AFB_1 alone ($p < .05$). [c]Values differ significantly from AFB_1 + GA1 ($p < .05$)

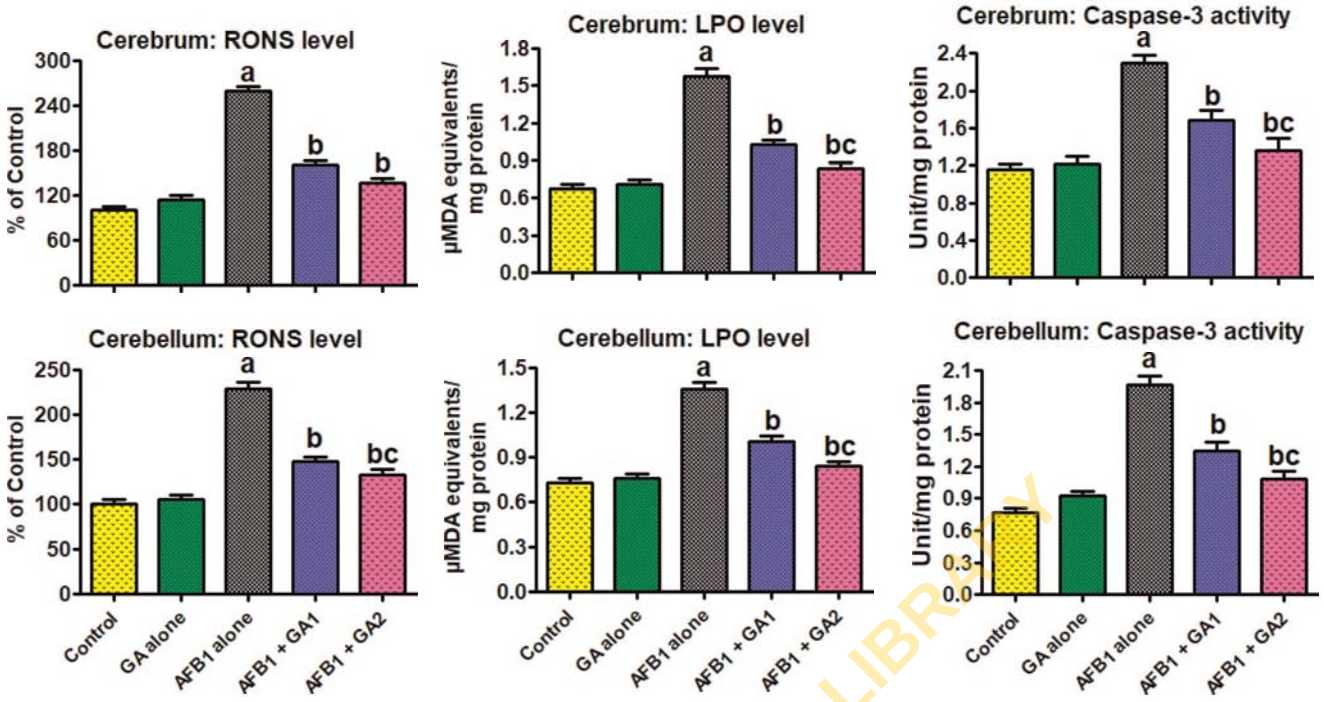

FIGURE 8 Impact of GA on RONS and LPO levels and caspase-3 activity in cerebrum and cerebellum of AFB_1-treated rats. GA1 and GA2 denote gallic acid at 20 and 40 mg/kg, respectively; AFB_1 denotes aflatoxin B_1 at 75 µg/kg. Data are presented as mean ± *SD* for 10 rats per group. AFB_1, aflatoxin B_1; GA, gallic acid; LPO, lipid peroxidation; MDA, malondialdehyde; RONS, reactive oxygen and nitrogen species. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from AFB_1 alone ($p < .05$). [c]Values differ significantly from AFB_1 + GA1 ($p < .05$)

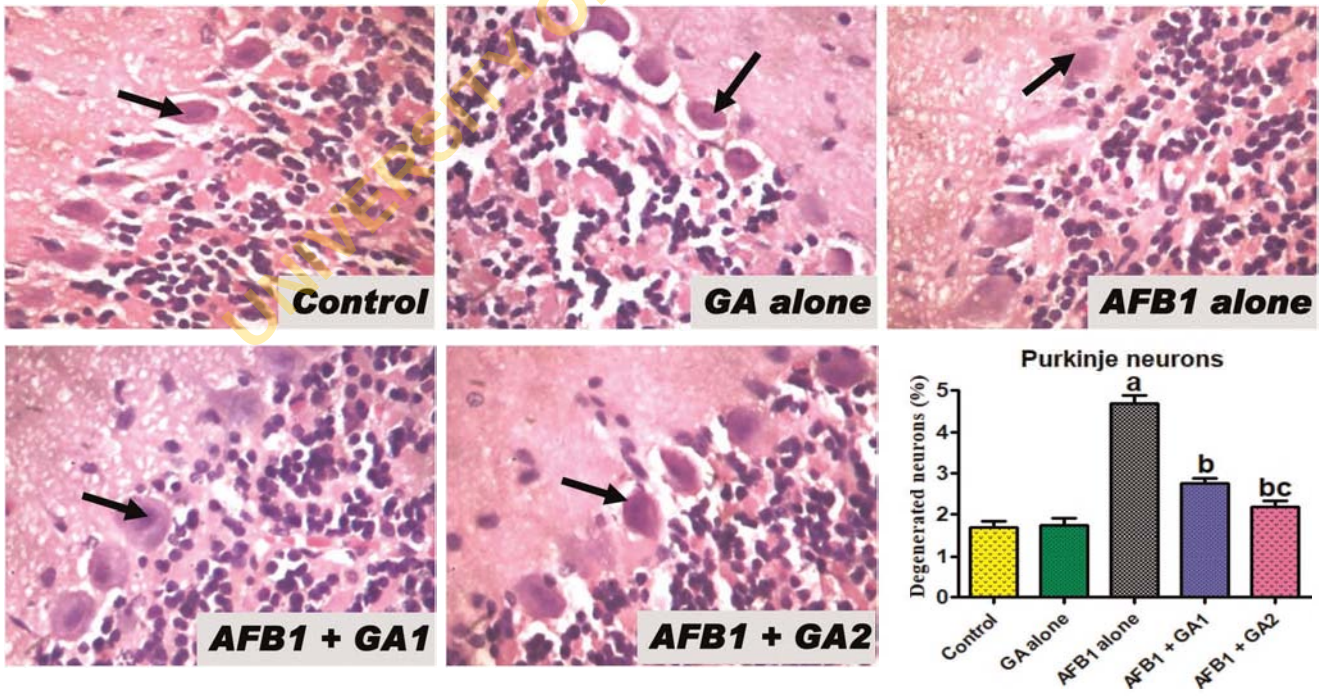

FIGURE 9 Representative photomicrographs and histomorphometry of the cerebellum. Control and GA alone sections showing normal structure. Sections from AFB_1 alone showing degenerated neurons with reduced nuclear material (black arrows) in the Purkinje cell layer of the cerebellum. Sections from rats cotreated with AFB_1 and GA appear similar to control. AFB_1, aflatoxin B_1; GA, gallic acid

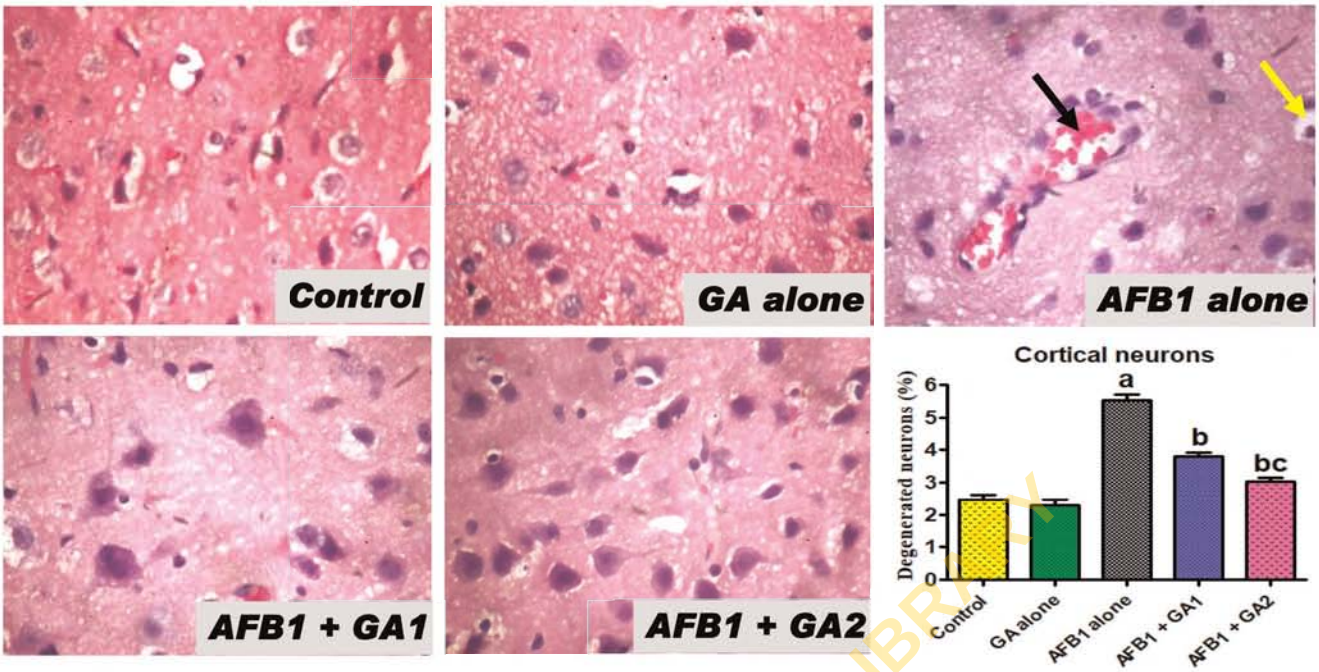

FIGURE 10 Representative photomicrographs and histomorphometry of the cerebral cortex. Control and GA alone sections showing normal structure. Sections from AFB_1 alone showing degenerated neurons with mild vacuolation of the neuropil (yellow arrow), onset of pyknosis, and marked congestion (black arrow) in the cerebral cortex. Sections from rats cotreated with AFB_1 and GA appear similar to control. AFB_1, aflatoxin B_1; GA, gallic acid

during physical activities.[48] This observation implies a deficiency in the coordination between the muscle and the nervous system in rats intoxicated to AFB_1 alone. Moreover, the decrease in total time mobile, maximum speed, distance traveled, and the ability to sustain a straight path in rats exposed to AFB_1 alone is indicative of locomotor inadequacies triggered by AFB_1. The boost in these motor and locomotor actions in rats cotreated with GA obviously revealed the abrogating effect of GA on AFB_1-induced neurobehavioral insufficiencies in the rats.

Anxiogenic-associated behaviors like increased freezing, geotaxis, frequency of urination, and fecal bolus discharge are often presented by animals during exposure to stressors.[34,35,49] The rats intoxicated with AFB_1 alone in the current study showed these anxiogenic-related behaviors, thus indicating a condition of anxiety and fear in the AFB_1-treated rats. The decrease in the freezing time, negative geotaxis, frequency of urination, and fecal bolus discharge in rats co-treated with GA at 20 and 40 mg/kg evidently demonstrated attenuation of AFB_1-mediated anxiety-like behaviors by GA. Moreover, the organism acquires information about its spatial surroundings through exploration.[50,51] Hence, the reduction in the density of track plots together with higher heat maps intensity following exposure to AFB_1 revealed incompetence in spatial and exploratory behavior of the exposed rats. This is the first study to characterize exploratory and anxiogenic-like behaviors associated with AFB_1-mediated neurotoxicity. Conversely, the enhancement in exploratory fitness as shown by increased track plot densities and lesser heat maps intensity upon treatment with GA at 20 and 40 mg/kg in this study clearly suggests abatement of exploratory and spatial behavior disorder associated with AFB_1 exposure by GA.

Biochemical assays, namely AChE activity, antioxidant status, GSH, oxidative, and inflammatory stress parameters, were done using cerebrum and cerebellum samples to elucidate the mechanisms of neuroprotection offered by GA in AFB_1-exposed rats. Locomotor and novelty exploratory behaviors are mostly controlled by the cholinergic neurotransmission system in numerous organisms.[52] Normally, AChE terminates the synaptic neurotransmission by converting acetylcholine, an essential cholinergic neurotransmitter, to choline and acetate. Thus, the decreased AChE activity in AFB_1-exposed rats in the current study indicates suppression of its effectiveness to hydrolyze acetylcholine. Backlog of acetylcholine at the synaptic nerve due to reduced AChE activity reportedly downregulates and desensitizes cholinergic receptors,[53] which eventually diminishes the normal neuronal function in the treated rats. Thus, the AFB_1-induced diminution in the AChE activity may be connected with locomotor, motor, and exploratory insufficiencies displayed by rats exposed to AFB_1 alone in this investigation. The boost in both AChE and behavioral activities following treatment with GA indicates its beneficial role in the enhancement of cholinergic neurotransmission in the treated rats.

The marked reduction in CAT, SOD, GPx, and GST activities in the cerebellum and cerebrum of rats administered AFB_1 alone showed that the cellular antioxidant defense mechanisms were compromised. This may subsequently result in cellular oxidative stress due to RONS buildup in the brain of the exposed rats. GSH is

an important nonenzymatic antioxidant largely responsible for the maintenance of thiol groups on membrane proteins as well as acts as a substrate in the detoxification of electrophilic compounds by GST and degradation of peroxides by GPx. It is a well-known marker of cellular detoxification and oxidative stress status.[54,55] The significant reduction in the cerebellar and cerebral GSH level in rats exposed to AFB_1 alone in this investigation indicates its excessive consumption to combat cellular oxidants. The decreased GST activity in AFB_1-treated rats may interfere with free-radical detoxification pathway by the enzyme. However, cotreatment with GA at 20 and 40 mg/kg abated AFB_1-induced reduction in antioxidant enzyme activities and GSH level in cerebrum and cerebellum of the rats. The restoration of antioxidant status due to GA treatment may be attributed to its antioxidant activities in the cerebellum and cerebrum of the rats.

Moreover, induction of oxidative stress mechanism following exposure to xenobiotics is associated with oxidation of cellular lipid components, leading to the formation of malondialdehyde (MDA) and 4-hydroxynonenal,[56] which subsequently interact with nucleic acids and proteins.[57] The increased level of RONS and MDA, a marker of LPO, in rats exposed to AFB_1 alone therefore suggests oxidative damage in the cerebellum and cerebrum of the rats. The abatement in RONS and LPO levels in rats administered both AFB_1 and GA in the current study signifies that GA cotreatment effectively attenuated RONS production but boosted cellular antioxidant defense capability, thereby inhibiting oxidative damage in the rats. The protective influence of GA on AFB_1-mediated neurotoxicity may be linked to its intrinsic antioxidant potential of augmenting antioxidant status and suppression of oxidative stress.

Further, the contribution of anti-inflammatory properties of GA in the neuroprotection was probed by assaying cerebellar and cerebral MPO activity, levels of NO, IL-1β, TNF-α, and IL-10. The normal activation of inducible nitric oxide synthase to synthesize NO and cytokines during inflammatory response in the cell is principally regulated by TNF-α.[58] However, excessive NO production results in depletion of antioxidant status, nitrosative stress, and cellular damage. Also, cellular activation of MPO is associated with increased hypochlorous acid production, which additionally harms the cells.[59] Therefore, elevated MPO activity and levels of pro-inflammatory biomarkers, namely NO, TNF-α, and IL-1β with diminution of anti-inflammatory cytokine IL-10 in the cerebellum and cerebrum of rats administered AFB_1 per se obviously evidenced a condition of inflammation and nitrosative stress. However, the abatement of these inflammatory parameters and an increase in IL-10 following administration of GA revealed the role of the anti-inflammatory mechanism of GA in lessening AFB_1-mediated neurotoxicity in the rats.

Finally, the assessment of caspase-3 activity and histological features of cerebellum and cerebrum was carried out to further expound the influence of GA on AFB_1-induced cell death and tissue damage in the treated rats. Caspase-3 is normally prompted to accomplish specific cleavage of proteins in the apoptotic cell death pathway.[60,61] The increase in caspase-3 activity in the cerebellum and cerebrum of rats exposed to AFB_1 alone suggests its activation in the apoptotic pathway. However, the reduction in the caspase-3 activity in rats cotreated with AFB_1 and GA demonstrates the anti-apoptotic role of GA by inhibiting the downstream executioner protease. Histopathological and morphormetrical findings revealed that GA treatment effectively attenuated AFB_1-induced neuronal degeneration in the cerebral cortex and Purkinje cell layer of the cerebellum, thus substantiating the neuroprotective role of GA in AFB_1-treated rats.

Collectively, undue exposure to AFB_1 caused neurotoxicity, which was considerably ameliorated by GA treatment. The neuroprotective mechanisms of GA are linked to enhancement of AChE activity and abatement of oxidative stress, inflammation, and caspase-3 activation in rats. Therefore, GA, a natural phytochemical with antioxidant and anti-inflammatory activities, may be a prospective therapeutic approach antidote for neurotoxicity caused by AFB_1 exposure. Also, plant sources of GA may be utilized as functional foods to mitigate the neurotoxic effect of AFB_1 in both animal production and humans.

ACKNOWLEDGMENT

This study was done without a specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The original data and materials of the current study are available with the corresponding author and would be made available on justifiable request.

ORCID

Isaac A. Adedara http://orcid.org/0000-0002-5997-178X
Solomon E. Owumi http://orcid.org/0000-0002-4973-0376

REFERENCES

[1] B. R. Rushing, M. I. Selim, *Food Chem. Toxicol.* **2019**, *124*, 81.
[2] A. Cimbalo, M. Alonso-Garrido, G. Font, L. Manyes, *Food Chem. Toxicol.* **2020**, *137*, 111161.
[3] Z. Xue, Y. Zhang, W. Yu, J. Zhang, J. Wang, F. Wan, Y. Kim, Y. Liu, X. Kou, *Anal. Chim. Acta* **2019**, *1069*, 1.
[4] IARC, *Monographs on Aflatoxins*, 82, IARC Press, Lyon **2000**, p. 171.
[5] S. Marin, A. J. Ramos, G. Cano-Sancho, V. Sanchis, *Food Chem. Toxicol.* **2013**, *60*, 218.
[6] N. Benkerroum, *Int. J. Environ. Res. Public Health* **2020**, *17*(4), 1215.
[7] M. McLean, M. F. Dutton, *Pharmacol. Ther.* **1995**, *65*, 163.
[8] T. R. Van Vleet, P. J. Klein, R. A. Coulombe Jr., *J. Toxicol. Environ. Health A* **2001**, *63*, 525.
[9] I. A. Adedara, M. K. Nanjappa, E. O. Farombi, B. T. Akingbemi, *Food Chem. Toxicol.* **2014**, *65*, 252.
[10] O. A. Oyelami, S. M. Maxwell, K. A. Adelusola, T. A. Aladekoma, A. O. Oyelese, *Mycopathologia* **1995**, *132*, 35.
[11] H. Qureshi, S. S. Hamid, S. S. Ali, J. Anwar, A. A. Siddiqui, N. A. Khan, *Med. Mycol.* **2015**, *53*, 409.
[12] T. S. Wu, Y. C. Cheng, P. J. Chen, Y. T. Huang, F. Y. Yu, B. H. Liu, *Chemosphere* **2019**, *217*, 905.

[13] T. Kihara, T. Matsuo, M. Sakamoto, Y. Yasuda, Y. Yamamoto, T. Tanimura, *Toxicol. Sci.* **2000**, *53*, 392.
[14] T. Tanaka, S. Mizukami, Y. Hasegawa-Baba, N. Onda, Y. Sugita-Konishi, T. Yoshida, M. Shibutani, *Toxicology* **2015**, *336*, 59.
[15] N. S. Souto, A. Claudia Monteiro Braga, M. Lutchemeyer de Freitas, M. Rechia Fighera, L. F. F. Royes, M. Schneider Oliveira, A. F. Furian, *Nutr. Neurosci.* **2018**, *21*, 268.
[16] Z. I. Linardaki, F. N. Lamari, M. Margarity, *Neurochem. Res.* **2017**, *42*(*10*), 2743.
[17] N. G. Bahey, H. O. Abd Elaziz, K. K. Gadalla, *Tissue Cell* **2015**, *47*, 559.
[18] F. H. Fernandes, H. R. Salgado, *Crit. Rev. Anal. Chem.* **2016**, *46*, 257.
[19] A. Abdel-Moneim, S. M. A. El-Twab, A. I. Yousef, E. S. A. Reheim, M. B. Ashour, *Biomed. Pharmacother.* **2018**, *105*, 1091.
[20] Y. L. Liu, C. C. Hsu, H. J. Huang, C. J. Chang, S. H. Sun, A. M. Y. Lin, *Mol. Neurobiol.* **2020**, *57*, 96.
[21] S. A. Ramakrishnan, N. Angayarkanni, *Basics in Biochemistry for Professional Nursing*, BI Publications Private Limited, New Delhi, India **2007**, p. 24.
[22] S. Maya, T. Prakash, K. Madhu, *Neurochem. Int.* **2018**, *121*, 50.
[23] A. S. Akinrinde, O. E. Adebiyi, *Neurotoxicology* **2019**, *74*, 252.
[24] N. Samad, S. Jabeen, I. Imran, I. Zulfiqar, K. Bilal, *Metab. Brain Dis.* **2019**, *34*(*4*), 1091.
[25] B. Ogunlade, S. A. Adelakun, J. A. Agie, *Drug Chem. Toxicol.* **2020**, *24*, 1. https://doi.org/10.1080/01480545.2020.1754849
[26] G. F. Brum, H. Z. Rosa, D. R. Rossato, J. L. O. Rosa, V. G. Metz, L. H. Milanesi, M. E. Burger, *Neurotox. Res.* **2020**, *38*(*2*), 274.
[27] M. Yu, X. Chen, J. Liu, Q. Ma, Z. Zhuo, H. Chen, L. Zhou, S. Yang, L. Zheng, C. Ning, J. Xu, T. Gao, S. T. Hou, *Neurobiol. Dis.* **2019**, *124*, 67.
[28] X. Zhao, Q. Q. Zhi, J. Y. Li, N. P. Keller, Z. M. He, *Toxins* **2018**, *10*, 270. https://doi.org/10.3390/toxins10070270
[29] S. E. Owumi, I. A. Adedara, A. P. Akomolafe, E. O. Farombi, A. K. Oyelere, *Exp. Biol. Med.* **2020**, *245*, 1016.
[30] S. Owumi, E. S. Najophe, E. O. Farombi, A. K. Oyelere, *J. Food Biochem.* **2020**, e13316. https://doi.org/10.1111/jfbc.13316
[31] I. A. Adedara, A. O. Abolaji, U. F. Idris, B. F. Olabiyi, E. M. Onibiyo, T. D. Ojuade, E. O. Farombi, *Chem. Biol. Interact.* **2017**, *261*, 1.
[32] B. A. Motz, J. R. Alberts, *Neurotoxicol. Teratol.* **2005**, *27*, 529.
[33] O. Folarin, F. Olopade, S. Onwuka, J. Olopade, *Oxid. Med. Cell Longev.* **2016**, *2016*, 4860582. https://doi.org/10.1155/2016/4860582
[34] E. C. Sarro, R. M. Sullivan, G. Barr, *Neuroscience* **2014**, *258*, 147.
[35] C. B. Quines, S. G. Rosa, J. T. Da Rocha, B. M. Gai, C. F. Bortolatto, M. M. Duarte, C. W. Nogueira, *Life Sci.* **2014**, *107*, 27.
[36] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248.
[37] G. L. Ellman, K. D. Courtney, V. Andres Jr, Feather-Stone R. M., *Biochem. Pharmacol.* **1961**, *7*, 88.
[38] A. Claiborne *Handbook of Methods for Oxygen Radical Research* (Ed: A. R. Greewald), CRC Press, Boca Raton **1995**, p. 237.
[39] H. P. Misra, I. Fridovich, *J. Biol. Chem.* **1972**, *247*, 3170.
[40] J. T. Rotruck, A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, W. G. Hoekstra, *Science* **1973**, *179*, 588.
[41] W. H. Habig, M. J. Pabst, W. B. Jakoby, *J. Biol. Chem.* **1974**, *249*, 7130.
[42] D. J. Jollow, J. R. Mitchell, N. Zampaglione, J. R. Gillette, *Pharmacology* **1974**, *11*, 151.
[43] E. O. Farombi, J. G. Tahnteng, A. O. Agboola, J. O. Nwankwo, G. O. Emerole, *Food Chem. Toxicol.* **2000**, *38*, 535.
[44] I. A. Adedara, A. O. Abolaji, J. B. Rocha, E. O. Farombi, *Neurochem. Res.* **2016**, *41*, 1430.
[45] L. C. Green, D. A. Wagner, J. Glogowski, P. L. Skipper, J. S. Wishnok, S. R. Tannenbaum, *Anal. Biochem.* **1982**, *126*, 131.
[46] S. Granell, M. Gironella, O. Bulbena, J. Panés, M. Mauri, L. Sabater, L. Aparisi, E. Gelpí, D. Closa, *Crit. Care Med.* **2003**, *31*, 525.
[47] F. C. Osuagwu, O. Owoeye, O. G. Avwioro, O. O. Oluwadara, I. O. Imosemi, R. S. Ajani, A. A. Ogunleye, O. W. Oladejo, *Afr. J. Med. Med. Sci.* **2007**, *36*, 103.
[48] B. L. Riemann, S. M. Lephart, *J Athletic Train* **2002**, *37*, 71.
[49] I. A. Adedara, O. B. Fasina, M. F. Ayeni, O. M. Ajayi, E. O. Farombi, *Food Chem. Toxicol.* **2019**, *125*, 170.
[50] J. H. Gorny, B. Gorny, D. G. Wallace, I. Q. Whishaw, *Learning and Memory* **2002**, *9*, 387.
[51] V. P. Bingman, P. E. Sharp, *Behav. Cogn. Neurosci. Rev.* **2006**, *5*, 80.
[52] J. Day, G. Damsma, H. C. Fibiger, *Pharmacol., Biochem. Behav.* **1991**, *38*, 723.
[53] C. J. Koppen, B. Kaiser, *Pharmacol. Ther.* **2003**, *98*, 197 van.
[54] O. Zitka, S. Skalickova, J. Gumulec, M. Masarik, V. Adam, J. Hubalek, L. Trnkova, J. Kruseova, T. Eckschlager, R. Kizek, *Oncol. Lett.* **2012**, *4*, 1247.
[55] G. Teskey, R. Abrahem, R. Cao, K. Gyurjian, H. Islamoglu, M. Lucero, A. Martinez, E. Paredes, O. Salaiz, B. Robinson, V. Venketaraman, *Adv. Clin. Chem.* **2018**, *87*, 141.
[56] A. Ayala, M. F. Muñoz, S. Argüelles, *Oxid. Med. Cell Longev.* **2014**, *2014*, 360438.
[57] M. Shichiri, *J. Clin. Biochem. Nutr.* **2014**, *54*, 151.
[58] V. Baud, M. Karin, *Trends Cell Biol.* **2001**, *11*, 372.
[59] N. Anatoliotakis, S. Deftereos, G. Bouras, G. Giannopoulos, D. Tsounis, C. Angelidis, A. Kaoukis, C. Stefanadis, *Curr. Top. Med. Chem.* **2013**, *13*, 115.
[60] A. G. Porter, R. U. Jänicke, *Cell Death Differ.* **1999**, *6*, 99.
[61] I. N. Lavrik, A. Golks, P. H. Krammer, *J. Clin. Invest.* **2005**, *115*, 2665.

How to cite this article: Adedara IA, Owumi SE, Oyelere AK, Farombi EO. Neuroprotective role of gallic acid in aflatoxin B_1-induced behavioral abnormalities in rats. *J Biochem Mol Toxicol*. 2021;35:e22684. https://doi.org/10.1002/jbt.22684