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Research Article
Preventive Efficiency of *Chelidonium majus* Ethanolic Extract Against Aflatoxin B_1 Induced Neurochemical Deteriorations in Rats

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Abstract
Background and Objective: Aflatoxins affect many species including humans and animals, therefore the present study was designed to investigate the protective effect of *Chelidonium majus* Ethanolic Extract (CMEE) on neurotoxicity induced by Aflatoxin B_1 (AFB1) in rats. **Materials and Methods:** Four groups of male Albino rats were treated orally for 28 days as follows: (1) Control group was daily given DMSO-PBS buffer (1.0 mL per rat), (2) CMEE (300 mg kg^{-1}/day) dissolved in DMSO-PBS buffer, (3) AFB1 (80 µg kg^{-1}/day) dissolved in DMSO-PBS buffer and (4) Received daily AFB1 (300 mg kg^{-1}) in combination with CMEE (300 mg kg^{-1}). **Results:** CMEE exhibits antioxidant activity *in vitro* and neuroameliorative efficiency *in vivo* as its administration in combination with AFB1 succeeded significantly in down regulating the elevated levels of inflammatory and apoptotic markers and restoring the values of neurochemical markers (AChE-ase, dopamine and serotonin) that were deteriorated by AFB1 intake. **Conclusion:** In conclusion, the neuroprotective effect of CMEE may be mediated through its antioxidant and free radical scavenging activity that proved from the data of ferric-reducing power ability and DPPH radical scavenging activity.

Key words: Aflatoxin, neurotoxicity, *Chelidonium majus*, oxidative stress, inflammation, cortex, hippocampus

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aflatoxins are naturally-occurring mycotoxins that contaminate agricultural products[1]. Aflatoxins affect many species including humans and animals[2]. The Aflatoxin B_1 (AFB1) is a secondary metabolite that is produced by the *Aspergillus parasiticus* and *Aspergillus flavus* fungi and is found in crops, foods and feedstuffs as a natural contaminant[3]. AFB1 is the most potent hepatotoxin and carcinogen and has a variety of toxicities such as mutagenicity, genotoxicity and immunotoxicity[4]. AFB1 ingestion causes also damage to the heart, kidney[5], intestine, bladder, pancreas, bone and viscera[6]. Furthermore, AFB1 causes degeneration in the central and peripheral nervous systems by direct toxicity to both the neural cells and blood-brain barrier[7,8] through significant changes in biochemical parameters[9]. Besides, it causes an inflammatory reaction in human microglial cells that is potentially harmful to the homeostasis of the central nervous system which may increase susceptibility to neurodegenerative diseases[10] like Reye's syndrome (cerebral oedema with neuronal degeneration) in man[11] and horses[12].

Chelidonium majus L., greater celandine, is a plant that belongs to the Papaveraceae family[13]. *Chelidonium majus* L. contains various alkaloids, flavonoids and phenolic acids that exhibit a wide range of pharmacological activities, such as antioxidant[14], antimicrobial, antitumor[15], antiviral[16] and anti-inflammatory effects[17]. It showed also choleretic, diuretic, analgesic and spasmolytic effects[18,19]. Extracts from *Chelidonium majus* have been used also in the treatment of digestive dysfunction, liver diseases, skin disorders, rheumatoid arthritis, tuberculosis and asthma[18]. These multiple pharmacological effects of *Chelidonium majus* are achieved due to numerous alkaloids contents, such as berberine, chelidonine, coptisine, chelerythrine and homochelidonine as well as flavonoids, carotene, vitamin C and phenolic acid[13].

Therefore, the current study was conducted to evaluate the antioxidant and neuroprotective role of the ethanolic extract *of Chelidonium majus* against aflatoxin B_1-induced neurotoxicity. To achieve this objective, we studied the protective effects of this extract on some oxidative stress indices, inflammatory and apoptotic markers. We also examined acetylcholinesterase activity, serotonin and dopamine as selective indices of brain functions, in two brain regions (cortex and hippocampus) in Albino rats.

MATERIALS AND METHODS

Study area: This study was carried out in the Laboratories of Medical Physiology and Animal House at the National Research Centre during the period between December, 2017-February, 2019.

Chemicals and plant extraction: Dimethyl Sulfoxide (DMSO) was purchased from Heiltropfen Co. Germany while AFB1 was purchased from the Cayman Chemical Co. (USA) and dissolved in dimethyl sulfoxide-buffer mixture (DMSO/PBS buffer, pH 7.2) before use. Other solvents and chemicals used were either analar or of a high analytical grade and obtained from the stores of the National Research Centre.

Dry stem branches of *Chelidonium majus* were obtained from Abd El-Rahman Harraz (Bab El-Khalk zone, Cairo, Egypt). The plant is carrying the taxonomic serial number 501481 and is identified in the Department of Botany, Faculty of Science, Cairo University, Egypt. The plant was ground, soaked in ethanol (70%) at a ratio of 1:5 (w/v) and kept at room temperature for 2 days in a closed container with continuous shaking, then filtered through a sterile filter paper under vacuum, finally, the ethanol was evaporated and then freeze-dried to remove water residues[20]. The resulting *Chelidonium majus* Ethanolic Extract (CMEE) yield was calculated as g/100 g crude powder and stored at -20°C until further use.

Total phenolics content: Total phenolic content was determined in the CMEE using Jayaprakasha *et al.*[21] methods. Triplicate measurements were made and the total phenolic content was estimated from the standard curve as catechin equivalent.

Radical scavenging activity (RSA): Determination of radical scavenging activity of CMEE by 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay was carried out following the method described by Nogala-Kalucka *et al.*[22]. The RSA was calculated as the percentage of the scavenged DPPH for three measurements.

Reducing power determination: The ferric-reducing power of CMEE was determined according to the method described by Sethiya *et al.*[23]. The reducing power of the extract was calculated as equivalent to ascorbic acid from the standard curve of ascorbic acid.

GC/MS identification of the chemical composition of the extract: The chemical composition of the ethanolic extract was identified using LC/MS/MS,4000 Qtrap Applied Biosystems, Waters Corporation, Milford, MA, USA, (Quadrupole/linear ion trap mass spectrometer) and the liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) system coupled with electrospray ionization-mass spectrometer (ESI-MS/MS) detector[24,25].

Experimental design: Thirty-two male Albino rats weighing 150-170 g were purchased from the animal colony of the Research Institute of Ophthalmology, Egypt. The animals were maintained on the standard laboratory diet and water *ad libitum* for a week before starting the experiment for acclimatization. The experimental protocol was approved by the Animal Care and Experimental Committee, National Research Centre (FWA 00014747), Cairo, Egypt.

The animals were divided into 4 groups (8 rats each) and treated daily for 28 days as follows:

- Control group, animals were orally administrated with DMSO-PBS buffer (1.0 mL per rat)
- Animals were orally given CMEE (300 mg kg^{-1}) dissolved in DMSO-PBS buffer[26]
- Animals were orally given AFB1 (80 µg kg^{-1}) dissolved in DMSO-PBS buffer[27]
- Animals received both AFB1 (80 µg kg^{-1}) and CMEE (300 mg kg^{-1}) together

Tissue sampling: At the end of the experimental period, the animals were sacrificed by sudden decapitation and the brain of each animal was rapidly dissected out onto an inverted ice-cold glass Petri dish. Each brain was anatomized to obtain the regions of the cortex and hippocampus, finally, the brain areas were frozen at -80°C until homogenization. A certain weight of each brain area was then homogenized in Tris-HCl buffer (pH 7.4) to give 10% w/v homogenate, using an ultrasonic homogenizer. The homogenate was centrifuged at 5000 rpm at 0°C for 15 min and the supernatant was used for the determination of oxidative stress markers, inflammatory cytokines levels and acetylcholinesterase activity. The remaining parts of the cortex and hippocampus were weighed and homogenized in 0.1 M perchloric acid (containing 3, 4-dihydroxybenzylamine) to give a concentration of 25 ng mL^{-1} then centrifuged for 10 min at 3600 rpm. The obtained supernatant was then filtered through 0.25 mm nylon filters, Millipore, USA and used for the determination of dopamine and serotonin.

Biochemical determinations: MDA level which is the product of lipid peroxidation was determined following the chemical method described by Ruiz-Larrea *et al.*[28] based on MDA reaction with thiobarbituric acid which forms a pink complex that can be measured photometrically. The activities of Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) enzymes were determined using reagent kits obtained from Biodiagnostic Co., Giza, Egypt. Nitric Oxide (NO) and Glutathione (GSH) levels were also assessed using reagent kits obtained from Biodiagnostic Co., Giza, Egypt.

Enzyme-Linked Immunosorbent Assay (ELISA) technique was used for the determination of Tumour Necrosis Factor-alpha (TNF-α), Interleukin-1 beta (IL-1β), caspase-3 and CD4 levels using rats' reagent (ELISA)-kits purchased from SinoGeneClon Biotech Co., Hangzhou, China.

Colorimetric determination of acetylcholine esterase (AChE) activity was carried out in the concerned brain areas by the method of Gomaa *et al.*[29], while the dopamine and serotonin levels were determined using HPLC (Waters, Melford, USA) and using an electrochemical detector following the method of Parrot *et al.*[30].

Statistical analysis: Multiple comparisons between means were carried out using one way ANOVA followed by post hock test (Duncan) at $p \geq 0.05$[31] using Statistical Analysis System (SAS) program software, Copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

RESULTS

The resulting *Chelidonium majus* ethanolic extract (CMEE) yield was 13 g/100 g crude powder and the determination of total phenolic content in the CMEE revealed that CMEE contains16.46±1.70 mg g^{-1}.

LC/MS analysis revealed the identification of 32 compounds in CMEE including organic acids, unsaturated fatty acids, phenolic compounds, volatile constituents and monosaccharides (Table 1).

The *in vitro* results showed the ability of the CMEE to scavenge 69±3% of DPPH• free radicals. The antioxidant properties are further confirmed in Fig. 1 which indicates the reducing power of CMEE which increased by increasing the concentration of the extract.

Table 2 depicts the effect of AFB1 and CMEE on the oxidative stress markers in brain regions. Significant increases in MDA and NO levels were detected in the cortex and hippocampus of the AFB1-treated group, while the GSH level and the activities of CAT, GPx and SOD enzymes showed significant decreases as compared to the control one. Administration of CMEE to the AFB1-intoxicated group induced significant improvement in all the mentioned

Table 1: LC/MS analysis of *Chelidonium majus* ethanolic extract

RT	Names	Formula	Area	Area sum (%)
4.912	Acetic acid, hydroxy-, ethyl ester	$C_4H_8O_3$	1068660.4	0.65
6.761	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	2019676.8	1.22
7.039	Acetaldehyde, hydroxy-	$C_2H_4O_2$	4194996.1	2.53
8.755	1,2,3-Propanetriol	$C_3H_8O_3$	3005097	1.82
9.359	Butane, 1,2:3,4-diepoxy-, (+/-)-	$C_4H_6O_2$	2394905.2	1.45
10.785	Acetic acid	$C_2H_4O_2$	6904804.3	4.17
11.008	2-Furancarboxaldehyde	$C_5H_4O_2$	1519991.6	0.92
11.208	Acetoxyacetic acid	$C_4H_6O_4$	1216938.4	0.74
12.035	Formic acid	CH_2O_2	5149305.6	3.11
13.824	Acetic acid ethenyl ester	$C_4H_6O_2$	1103245.2	0.67
14.12	Cyclopent-2-en-1,4-Dione	$C_5H_4O_2$	1590178.2	0.96
16.754	2-Furanmethanol	$C_5H_6O_2$	2008274	1.21
19.503	Aziridine, 2-(1,1-dimethylethyl)-3-methyl-, trans-	$C_7H_{15}N$	1158139	0.70
21.437	Propanoic acid, 3-(trimethylsilyl)-, ethyl ester	$C_8H_{18}O_2Si$	1279523.7	0.77
24.754	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	961938.81	0.58
26.766	Dihydroxyacetone	$C_3H_6O_3$	17226638	10.41
27.817	2,3-Pentanedione, 4-methyl-	$C_6H_{10}O_2$	1745695.1	1.05
28.639	Succindialdehyde	$C_4H_6O_2$	979407.43	0.59
28.705	4,4'-Biscyclohexanone, 2,2',6,6'-tetramethyl-	$C_{16}H_{26}O_2$	1014864.9	0.61
31.449	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	4581077.4	2.77
31.823	1,3-Isobenzofurandione	$C_8H_4O_3$	924945.06	0.56
33.877	Glycerin	$C3H_8O_3$	37839317	22.86
39.781	5-Hydroxymethylfurfural	$C_6H_6O_3$	21098441	12.75
40.953	Vanillin	$C_8H_8O_3$	1014156.1	0.61
41.11	Cyclopentanone, 2-(1-methylpropyl)-	$C_9H_{16}O$	1635576.3	0.99
41.907	3,6-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	1759606.8	1.06
42.222	2H-Pyran-2-methanol, tetrahydro-	$C_6H_{12}O_2$	1574980.4	0.95
42.548	2-Deoxy-D-galactose	$C_6H_{12}O_5$	1882631.3	1.14
43.418	Cyclohexane, 1,1'-[1-(2,2-dimethylbutyl)-1,3-Propanediyl] BIS	$C_{21}H_{40}$	910330.27	0.55
44.91	DL-Arabinose	$C_5H_{10}O_5$	2268575.6	1.37
49.242	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	23032628	13.91
49.889	Hexadecanoic acid	$C_{16}H_{32}O_2$	10476487	6.33

Table 2: Oxidative stress markers of brain regions (cortex and hippocampus) of control, AFB1 and CMEE-treated rats

Area	Parameters	Control	CMEE	AFB1	AFB1+CMEE
Cortex	MDA (μmol g^{-1})	4630±103	4740±118	6463±137*	5212±87#
	NO (nmol g^{-1})	0.812±0.018	0.856±0.022	1.21±0.031*	0.899±0.019#
	CAT (μmol/min/g)	5983±49	5818±46	4336±38*	5278±51#
	GPx (μmol/min/g)	18.97±0.95	19.48±0.93	14.04±0.81*	17.57±0.97#
	SOD (U g^{-1})	2772±57.3	2875±55.5	1681±17.8*	2270±23.7#
	GSH (μmol g^{-1})	4999±44.5	5098±46.6	4440±37.5*	4824±31.4#
Hippocampus	MDA (μmol g^{-1})	3856±41	3679±38	6035±54*	4187±33#
	NO (nmol g^{-1})	2.59±0.028	2.48±0.025	4.93±0.034*	3.12±0.022#
	CAT (μmol/min/g)	6061±55	6176±60	4055±39*	5570±41#
	GPx (μmol/min/g)	25.44±0.65	26.38±0.58	18.38±0.37*	23.04±0.41#
	SOD (U g^{-1})	3741±67	3792±63	2146±34*	3387±57#
	GSH (μmol g^{-1})	14008±63	14177±69	9824±48*	12880±51#

Data are presented as Mean±SEM and subjected to one-way ANOVA followed by post hoc test (Duncan) at $p\leq 0.05$, within the same row,*Significant vs. control group and #Significant vs. AFB1 group

parameters and reversed their values towards the values of the controls. Insignificant changes in oxidative stress markers were observed between the control group and those that received CMEE alone, indicating the safety of CMEE at the used dose.

The results obtained revealed that TNF-α, IL-1β, caspase-3 and CD4 were significantly increased in both the cortex and hippocampus of AFB1 group, while as animals received the CMEE alone showed insignificant changes in these parameters when both groups were compared with the control group. The animals that were given CMEE in combination with AFB1 showed significant decreases in TNF-α, IL-1β, caspase-3 and CD4 as compared to the AFB1-treated group (Table 3).

AFB1 intoxication inhibited ACh-ase activity (Fig. 2a) and significantly decreased dopamine (Fig. 2b) and serotonin (Fig. 2c) levels in the cortex and hippocampus, while

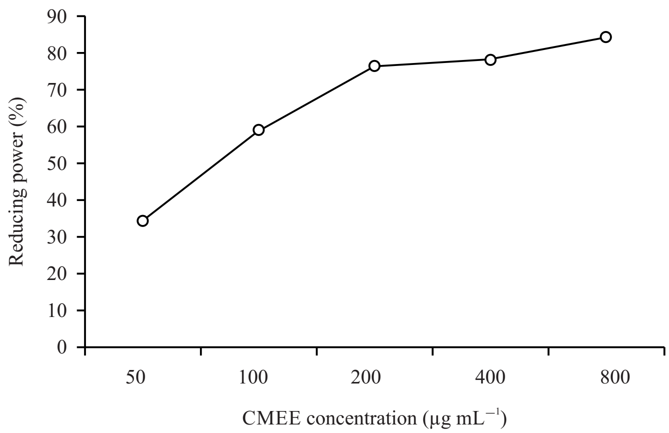

Fig. 1: Reducing power ability of *Chelidonium majus* ethanolic extract

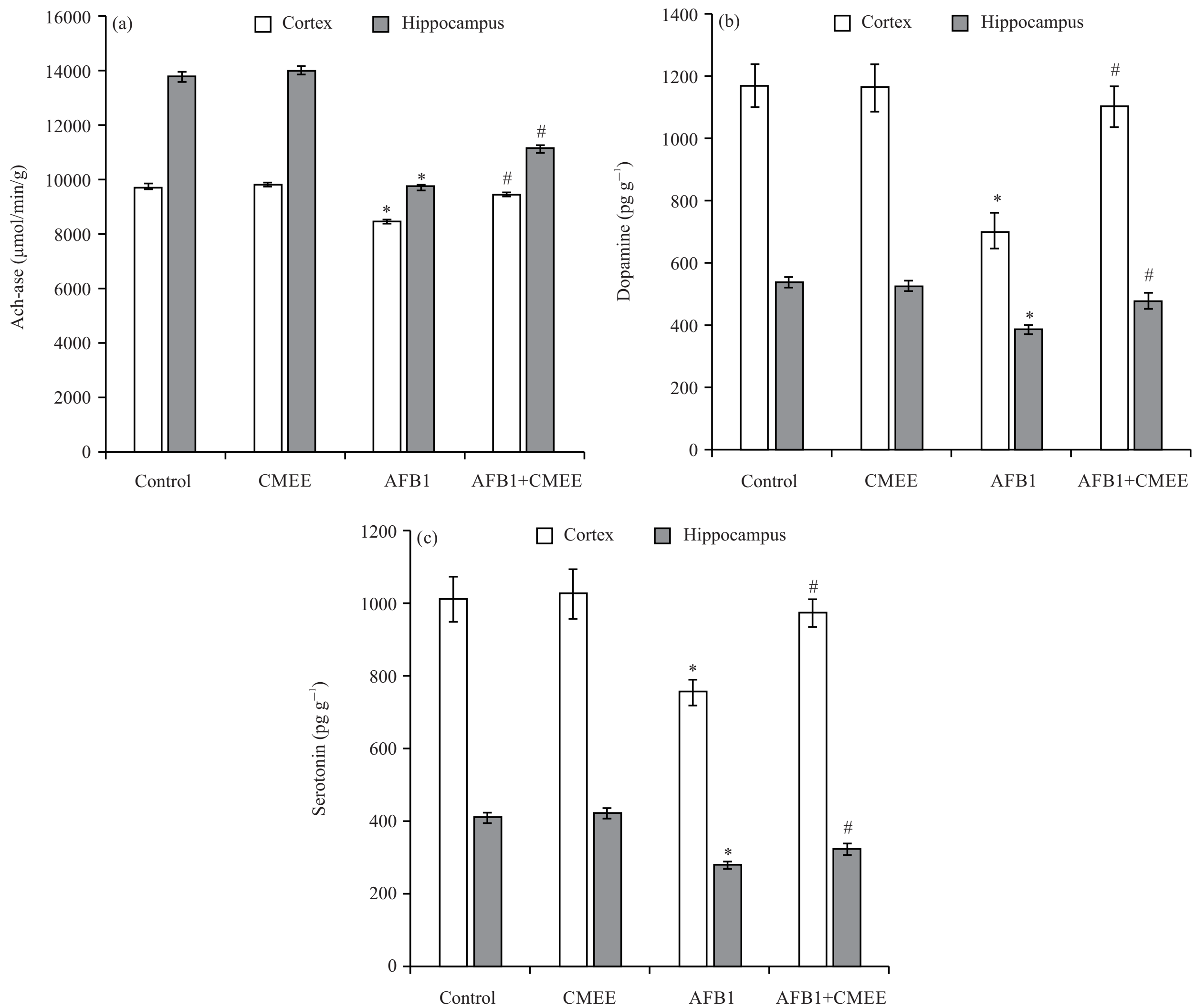

Fig. 2(a-c): Cortical and hippocampal, (a) ACh-ase activity, (b) Dopamine and (c) Serotonin levels of control, AFB1 and CMEE-treated rats

Data are expressed as Mean±SEM, *Significant vs. control group and #Significant vs. AFB1 group

Table 3: Levels of inflammatory cytokines and apoptotic markers in cortex and hippocampus of control, AFB1 and CMEE-treated animals

Area	Parameters	Control	CMEE	AFB1	AFB1+CMEE
Cortex	TNF-α (ng g^{-1} tissue)	13.20±0.41	12.88±0.44	17.81±0.53*	14.64±0.48#
	IL-1β (ng g^{-1} tissue)	128.33±3.7	126.95±4.5	182.29±6.1*	152.13±4.8#
	Caspase-3 (pg g^{-1} tissue)	14.13±1.07	12.67±0.85	23.05±2.44*	15.22±1.87#
	CD4 (U g^{-1} tissue)	37.4±1.8	34.5±2.1	62.6±2.8*	43.9±2.4#
Hippocampus	TNF-α (ng g^{-1} tissue)	24.99±1.99	23.91±2.3	38.43±3.7*	29.34±3.3#
	IL-1β (ng g^{-1} tissue)	147±4.2	141±3.8	296±6.6*	186±4.1#
	Caspase-3 (pg g^{-1} tissue)	71.1±3.8	67.8±4.6	96.8±6.2*	76.2±4.3#
	CD4 (U g^{-1} tissue)	58.6±2.3	56.7±2.2	105.7±3.1*	72.8±2.9#

Data are presented as Mean±SEM and subjected to one-way ANOVA followed by post hoc test (Duncan) at $p\leq 0.05$, within the same row,*Significant vs. control group and #Significant vs. AFB1 group

treatment with CMEE alone had no significant effect on the values of the mentioned parameters as compared to the control group. Treatment of AFB1- intoxicated rats with CMEE was found to be effective in restoring ACh-ase activity and levels of biogenic amines in these brain regions.

DISCUSSION

The results of the current study revealed that AFB1 intoxication resulted in a marked disturbance in the oxidative status of the studied brain regions. AFB1, known to be the most toxic of the aflatoxins[32]. Oxidative stress which is the main direct mechanism of aflatoxin or its metabolites[33] induces an excessive generation of free radicals and causes the production of high levels of Lipid Peroxidation (LPO) that may increase the susceptibility of the brain to neural damage. Lipid peroxidation is described as the peroxidation of unsaturated fatty acids of the cell membrane and organelles by free radicals[34]. The brain contains a great amount of polyunsaturated fatty acids and consumes about 20% of the body's oxygen, so it is particularly susceptible to oxidative damage. Moreover, the brain has a high rate of oxidative metabolism, although the activity of its antioxidant defence mechanism is relatively low[35,36].

The antioxidant enzymes (SOD, CAT and GPx) play an important role in the conversion of free radicals into harmless compounds[37,38], the significant deterioration in the activity of these enzymes, post-AFB1 ingestion, suggests the participation of free radical-induced oxidative cell injury in mediating the toxicity of AFB1. Our results agree with those obtained by Gugliandolo *et al*.[39], who observed that AFB1 administration significantly decreased antioxidant enzymes activities and increased LPO in the brains of experimental animals.

AFB1 is metabolized largely by the hepatic cytochrome P450 enzyme system, to form the Reactive Oxygen Species (ROS), AFB1-8,9-epoxides[40,41]. These epoxides may attack the critical molecules which include DNA, lipids and proteins with elevation in LPO levels and decreasing the endogenous antioxidants which ultimately lead to the impairment of cell function and cytolysis[42]. AFB1 and its metabolites are transported from the liver into the bloodstream, penetrate through the Blood Brain Barrier (BBB) and reach the brain via the metabolite transporter within oligodendrocytes, astrocytes, microglia and neurons cells[43] and trigger oxidative stress[44] causing damages to the brain tissues[45,46].

Other studies have suggested that AFB1-enhanced peroxidation and neurodegenerative disorders may be related to up-regulation of Inducible Nitric Oxide Synthase (iNOS) mRNA and increased Nitric Oxide (NO) production, a factor of endothelial relaxation, in rat astrocytes and microglial cells[7]. In addition, AFB1 activates nitric oxide and consequently affects brain microvascular endothelial cells which constitute the BBB involving astrocytes[9]. The significant increase in cortex and hippocampus NO content which is observed in the current study further confirms the pro-oxidative effect of AFB1 in brain tissues.

It is well established that antioxidants intake is an efficient way to raise and fortify endogenous defence systems due to their role in decreasing oxidative stress resulting in cytoprotection. In the current study, treatment with CMEE reduced lipid peroxidation and NO production and increased the values of SOD, CAT, GPx and glutathione in the brain cortex and hippocampus of AFB1-treated rats. These results indicated the antioxidant potential of CMEE and demonstrated its ability to protect the brain from AFB1 toxicity by reducing oxidative stress in brain tissues. The present study suggests that the antioxidant capacity of CMEE may relate to its bioactive component.

In the present study, elevated levels of TNF-α and IL-1β, pro-inflammatory cytokines, were found in the cortex and hippocampus of the AFB1 group. AFB1 can cause inflammatory reactions in human microglial cells which are potentially baleful to the homeostasis of the central nervous system and consequently increase susceptibility to neurodegenerative diseases[7]. AFB1 intake can trigger Toll-like

Receptors (TLR2), recognition receptors, and with activation of the NF-kB pathway result in a significant elevation in the secretion of TNFα and IL-1β in microglial cells[7]. Previously, Mehrzad *et al*.[10] have shown that AFB1 can activate rat microglial cells to produce significant amounts of proinflammatory cytokines at mRNA and protein levels. In addition, Mehrzad *et al*.[10] found that a low concentration of AFB1 induced intracellular ATP depletion and increased mRNA expression of many proinflammatory molecules such as MyD88, TLRs, NF-κB and C×Cr4, in the human microglial cell line.

The oxidative stress that induced by mycotoxins toxicity suppress astrocytes on which neurons are dependent for protective signals resulting in accelerating apoptosis-related pathways[47]. Apoptosis is a normally occurring cell death mechanism used to develop and maintain healthy tissues[48]. Many diseases can be developed due to apoptotic dysregulation, such as neurodegeneration, cancer and immunological diseases[49]. These apoptotic events can disturb the homeostasis, leading to the degeneration of the brain barrier and then the occurrence of neural damage[50]. In our study, we found a significant elevation in caspase-3 in both brain regions. Caspase-3 is an apoptotic marker, activated during cellular exposure to cytotoxic agents[51]. In this concern, Mehrzad *et al*.[7] observed in their *in vitro* study, that AFB1 exposure triggered inflammatory reactions and induced also a pro-apoptotic condition in murine pure primary astrocytes.

Our results supposed that CMEE has anti-inflammatory effects by inhibiting the release of TNF-α and IL-1β which may be influenced by the bioactive components in the extract. Several reports have demonstrated the anti-inflammatory ability of the main bioactive components of *Chelidonium majus*. It is found that stylopine which isolated from *Chelidonium majus* suppressed gene expression of Cyclooxygenase-2 (COX-2) and Inducible Nitric Oxide Synthase (iNOS) and consequently inhibited the production of prostaglandin E_2 and nitric oxide in Lipopolysaccharide (LPS)-treated RAW264.7 macrophages[17]. In another study conducted on LPS -treated murine RAW264.7 macrophages, some alkaloids isolated from *Chelidonium majus*, namely stylopine, methyl 2′-(7,8-dihydrosanguinarine-8-yl) acetate, norchelidonine, protopine, chelidonine, berberine and 8-hydroxydihydrosanguinarine, have been found to inhibit inductions of COX-2 and iNOS mRNA in dose-dependent manners[52].

CD4 is a monomeric glycoprotein expressed on approximately 60% of peripheral blood T lymphocytes[53,54]. It is suggested that CD4 increases the capacity of T cells for Major Histocompatibility Complex (MHC) class II-bearing cells[55,56]. The function of MHC class II molecules is the presentation of processed foreign antigens to $CD4^+$ T-lymphocytes[57]. Several reports suggested that CD4 participates in signal transduction during T cell activation[58,59]. We observed in our study a significant increase in CD4 in the cortex and hippocampus of rats given AFB1 alone, while treatments of AFB1 rats with CMEE returned CD4 values to those of the control rats. It is well confirmed that the Central Nervous System (CNS) is largely invaded by peripheral leukocytes in neuropathological states[60]. Brochard *et al*.[61] have shown that peripheral T cells invade the brain parenchyma at the site of neuronal injury in Parkinson Disease (PD) in humans and in experimental animals. Also, Brochard *et al*.[61] have demonstrated that this cell-mediated immune response contributes to the degeneration of Dopamine-containing Neurons (DNs) by a $CD4^+$ T cell through Fas/FasL cytotoxic pathway. These reports could explain the observed high CD4 levels in the cortex and hippocampus in response to damage induced by AFB1 in the AFB1 intoxicated group. On the other hand, the lowering effect of CMEE on CD4 levels in AFB1 intoxicated group may be attributed to the reduction in $CD4^+$ producing T cells as indicated by Lee *et al*.[62], who found that CMEE could decrease the number of $CD4^+$ T cells in the spleen and lymph node in collagen-induced arthritis model mice and suggested that CMEE might suppress the immune response by lowering $CD4^+$ T cells and enhancing $CD8^+$ T cells.

In the current study, AFB1 intoxication inhibited AChE activity in both brain regions. This finding was confirmed in previous studies conducted by Scafuri *et al*.[63] and Linardaki *et al*.[64] which indicated that AFB1 can inhibit the activity of AChE of rat whole brain, hippocampus and cerebellum. The mechanisms behind the inhibitory effect on AChE activity could be due to the blocking accession of the substrate to the active site of the enzyme and/or the binding to its peripheral site[65].

The entrance of AFB1 and/or its metabolite into the brain through BBB results in biochemical and pathological changes[66] leading to brain dysfunction. In the current study, a significant decrease was noticed in dopamine and serotonin content in the cortex and hippocampus due to AFB1 neurotoxicity. These neurotransmitters are known to play an important role in brain function. Significant decreases in dopamine and serotonin values were noticed also in previous studies conducted in rats[67] and chicken[66]. The lowering effect of AFB1 on these neurotransmitters may be due to the disruption of mechanisms that facilitate the synthesis of serotonin from tryptophan[68] and dopamine from tyrosine[69]. Other mechanisms have been suggested, including oxidative stress[69] which causes membrane damage that affects neuron integrity. Growing evidence suggests that neuroinflammatory processes accelerate the progression of death of Dopamine-containing Neurons (DN) [69-71] through $CD4^+$ Th FasL-mediated activation of microglial cells that could participate in the inflammatory reaction and DN degeneration[72].

The present study demonstrated that CMEE is effective in reducing lipid peroxidation and inflammation and improving the antioxidant enzymes and glutathione in the cortex and hippocampus. Additionally, CMEE could restore brain function that appeared from restoring AChE-ase, dopamine and serotonin values that decreased by AFB1 intake. These results indicated that CMEE exerted its neuroprotective activity through its antioxidant ability that proved from the data of reducing power ability and DPPH radical scavenging activity. This might be attributed to the ability of CMEE to prevent depletion of antioxidant enzymes and GSH and increase the endogenous defensive capacity of the brain to resist the free radicals and inflammation induced by AFB1 toxicity, this leads to improvement of neuron integrity and prevents its destruction and consequently improves brain function and hence restoring the synthesis and function of the neurotransmitter. The neuroprotective potential of CMEE may be due to the numerous and diverse phytochemicals present in the extract. In the present study, LC/ MS analysis revealed the identification of 32 compounds in CMEE including organic compounds, unsaturated fatty acids, volatile constituents, monosaccharides and nutrients. Furthermore, CMEE contains 13 phenolic compounds which are described as having an antioxidative capacity *in vitro* and *in vivo* [73]. Researchers have described the anti-inflammatory activity of unsaturated fatty acids. The fatty acid, n-hexadecanoic acid has been found to inhibit phospholipase A (2). The inhibition of phospholipase A (2) is considered as one of the ways that control inflammation[74]. Bhattacharjee *et al.*[75] reported the protective effect of the fatty acid cis-9 octadecenoic acid (Oleic acid) against oxidative stress-mediated hepatic and cardiac injuries. 5-hydroxymethylfurfural is known as an antioxidant and it was found to accelerate wound healing through improvement in inflammation, increased angiogenesis and collagen production and promoted re-epithelialization[76]. Wei *et al.*[77] reported the antioxidative activity of the volatile constituent, 2-furan methanol. CMEE also contains nutrients such as vanillin which has antidepressant, antioxidant and anti-glycating properties. It has also protective effects on cardiotoxicity induced by doxorubicin[78]. 4H-Pyran-4-One 2,3-Dihydro-3,5-Dihydroxy-6-Methyl component was found to ameliorate reproductive toxicity and decrease malondialdehyde and nitric oxide levels in cadmium chloride-intoxicated rats[79]. Another compound that may also play a role in the CMEE activity is acetic acid, this organic acid is known for its anti-inflammatory, hypoglycemic and hypotensive effects[80]. The current study confirms that *Chelidonium majus* ethanolic extract builds a defence battery against AFB1-induced oxidative stress in rats' brain tissue. To appreciate the ameliorative potency of this extract in minimizing overall AFB1-induced immune-inflammatory and neurochemical deterioration, experimental antioxidant therapy in laboratory animals is being explored. If the experimental models have positive outcomes, it might be worth further study to use them in integration with the existing clinical interventions approved.

CONCLUSION

In conclusion, the current study indicated that AFB1 ingestion resulted in severe neurotoxic action. Treatment with CMEE in combination with AFB1 could produce neuroprotective action via its antioxidant and free radical scavenging activity which may be related to its higher contents of bioactive constituents. Therefore, CMEE may be a candidate for the prevention and treatment of neurotoxicity resulting from the ingestion of aflatoxins contaminated diets.

SIGNIFICANCE STATEMENT

This study explores the possible ameliorating efficiency of *Chelidonium majus* ethanolic extract against AFB1-resulted neuron deterioration rats. This study will help toxicology researchers to uncover that this herb may be beneficial in the improvement of brain status as it is considered the most important organ in the human body. Thus, a new theory on this herb may be arrived at.

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