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ORIGINAL ARTICLE

Neuroprotective Effect of *Cucurbita pepo* in Lipopolysaccharide-Induced Toxicity in C57BL/6 Mice

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ABSTRACT

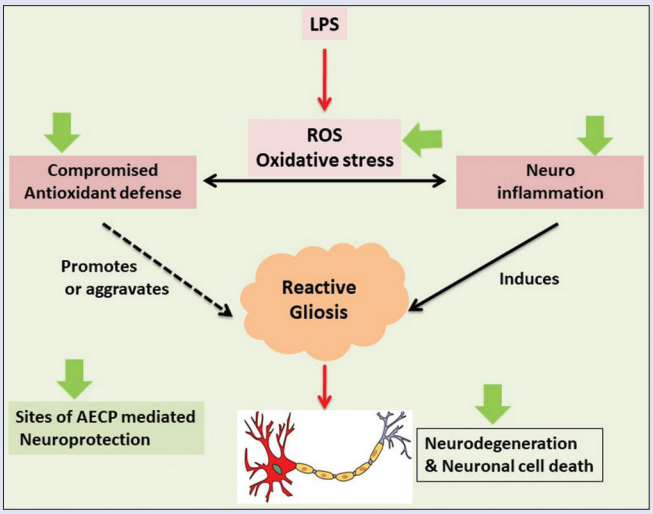
Background: Although the powerful scientific studies discover *Cucurbita pepo* probable in the treatment of various ailments, *Cucurbita pepo* being employed as food material in various part of the world, there is indeterminate in the neuroprotective potential of *Cucurbita pepo* in LPS induced neuroinflammation experimental model. **Aim and Objectives:** This study is intended to examine the protective effect of *Cucurbita pepo* against LPS induced neuro-inflammation in C57BL/6 mice. **Materials and Methods:** A single dose intraperitoneal (i.p) injection of lipopolysaccharide liquefied in saline was given to C57BL/6 mice. (n=10 per group). Aqueous extract of *Cucurbita pepo* (AECP) at a concentration of (100 mg/kg b.w.) was administered orally to C57BL/6 mice 1 hour before LPS induction (7 days) and continuous till 30 days (n=10 per group). **Results:** Levels of enzymatic antioxidants such as catalase (43.26±4.61 %) and superoxide dismutase (43.16±3.82%) restored decidedly and non-enzymatic antioxidants GPx and GSH restored up to 20.81±4.22 and 58.28±2.44 percentage respectively upon AECP treatment. Oxidative stress markers NO and LPO abridged to 57.64±3.17 and 53.25±2.53 percentage compared to LPS induction group. AECP treatment lessened the protein expression level of proinflammatory cytokines Tumor Necrosis Factor-α (TNF-α), Interleukin 1 beta (IL1-β) and Interleukin 6 (IL-6) significantly ($P < 0.05$) detected by ELISA. Signs of prolong inflammation caused higher expression of isoforms of nitric oxide synthases genes (eNOS, nNOS and iNOS) signifies NO productivity. In the cortex of LPS challenged mice, AECP significantly (P <0.05) condensed the LPS persuaded expressions of eNOS (1.98±0.41 fold), nNOS (1.74±0.26 fold) and iNOS (1.81±0.52 fold) genes. Also, oral administration of AECP significantly ($P < 0.05$) reduced the expression of ionized calcium binding adaptor molecule (Iba-1) in LPS induced mice brain cortex (30.37%), were additional supports the anti-inflammatory potential of AECP. **Conclusion:** In summary AECP displays antioxidant and anti-inflammatory potential against LPS induced neuroinflammation. However, the mechanistic insights of *Cucurbita pepo* in neuroprotection potential have explained in detail.

Key words: *Cucurbita pepo,* ionized calcium-binding adaptor molecule-1, lipopolysaccharide, neuroinflammation, nitric oxide, oxidative stress

SUMMARY

- Aqueous extract of *Cucurbita pepo* (AECP) applies antioxidant effect against lipopolysaccharide (LPS)-persuaded oxidative stress in mice brain
- AECP restores histological changes and reduced cell atrophy induced by LPS-induced oxidative stress
- AECP treatment diminishes LPS-induced neuroinflammation in mice brain.

Abbreviations used: AECP: Aqueous extract of *Cucurbita pepo*; LPS: Lipopolysaccharide; SOD: Superoxide dismutase; CAT: Catalase; GSH: Reduced glutathione; GPX: Glutathione peroxidase; TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; eNOS: Endothelial nitric oxide synthase, nNOS: Neuronal nitric oxide synthase; iNOS: Inducible nitric oxide synthase; Iba-1: Ionized calcium-binding adaptor molecule-1; PD: Parkinson's disease; AD: Alzheimer's disease; HD: Huntington's disease; ALS: Amyotrophic lateral sclerosis; LPS: Lipopolysaccharide; NO: nitric oxide; CNS: Central nervous system; NF-kB: Nuclear factor-kappa B; BSA: Bovine serum albumin; DAB: Diaminobenzidine tetrahydrochloride; H_2O_2: Hydrogen peroxide; DMSO: Dimethyl sulfoxide; b.wt: Body weight; mg: Milligram; HRP: Horseradish peroxidase; TBARS: Thiobarbituric acid reactive substances; LPO: Lipid peroxidation; TBS-T20: Tris-buffered saline–Tween 20; O/N: Overnight; SEM: Standard error of mean.



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INTRODUCTION

Neuroinflammation is extremely observed as mediator of neurodegenerative situations such as Parkinson's Disease (PD), Alzheimer's Disease (AD), Huntington's Disease (HD), and Amyotrophic Lateral Sclerosis (ALS).[1] Neuroinflammation can be acute and chronic, acute neuroinflammation is reported to be protective, while chronic neuroinflammation is harmful and pathologic.[2,3] Pathogenic response during both acute and chronic inflammation in nervous system were

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resident macrophages of microglia and astrocytes are being the main cellular phenotypes involved in neuroprotection.[4,5] Accumulating indications reports activation of microglia and astrocytes during neuroinflammation which is both protective and detrimental based on the setting of its duration of activation[6-8] Moreover, number of studies documented that lipopolysaccharide mediated intoxication (LPS) activates microglia and astrocyte in central nervous system resulting in release of pro-inflammatory gaseous cytokines called nitric oxide (NO), protein cytokines like Tumor Necrosis Factor alpha (TNF-α), Interleukin 1β (IL)-1β and Interleukin 6 (IL-6). These molecules identified as key mediators in most of neurodegenerative disorders such as AD, PD, ALS and HD.[9-12]

Microglia cells deliver primary defense for LPS-mediated inflammation via both innate and adaptive immune responses in the central nervous system (CNS).[13,14] Although microglial activation offers defenses, documenting reports means chronic activation and noxious stimuli can result in dysfunctional microglial phenotype leading to exacerbation of neuroinflammation and neuronal demise as gotten in AD,[15] multiple sclerosis,[16] and PD.[17] Especially, LPS exposure mediates activation of nuclear factor-kappa B via releasing its inhibitor IkappaB (IkB), thereby mediating expression of aforesaid pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and NO that can perpetuate inflammation and neuronal cell death.[18,19] Undeniably, mechanism of LPS-induced neuroinflammation and cell death remains undecided,[20] and hence, many approaches were explained to regulate the toxic effects of infection and inflammation in brain pathologies and in neurodegenerative conditions. Here, we assume that LPS-induced ROS and oxidative stress[21,22] might serve as contributing factor in the setting up of neuroinflammation upon LPS exposure and hence countering the same via natural agents will pave way for neuroprotective avenues.[5,23]

Recently, scientific studies mainly emphasis the neuro-protective role of natural resources, exclusively pays more attention on mechanism of action. Pharmacological effect of food supplements was always attributed by its bioactive metabolites.[24] In folk medicine, a vegetables were usually used to ease many ailments. Mainly, *Cucurbita pepo* is used as a therapy to cure hepatorenal, gastroenteritis, and brain anomalies. Earlier, preliminary studies on neuroprotective potential of *C. pepo* fruit peel against CCl_4-induced neurotoxicity suggested that there is a useful alteration in the level of antioxidant enzymes in rat model.[25]

Here, we have examined the protective role of *C. pepo* in LPS-induced neuroinflammation; we inspected the effect of *C. pepo* in LPS-induced oxidative stress, microglia activation, and neuroinflammation *in vivo* using C57BL/6 mice model.

MATERIALS AND METHODS

Chemicals and their sources

Bovine serum albumin (BSA), diaminobenzidine tetrahydrochloride (DAB), hydrogen peroxide (H_2O_2), agarose, and dimethyl sulfoxide were procured from standard vendor company Sigma Chemical Company (St. Louis), USA. Materials for Q-polymerase chain reaction (PCR), namely C-DNA kit and diethyl pyrocarbonate water, were procured from Vazyme Biotech Co., Ltd., Nanjing, P. R., China. Gene-specific primers for Q-PCR were obtained from Vazyme Biotech Co., Ltd., Nanjing, P. R., China. All the other chemicals employed were of analytical grade unless otherwise specified.

Preparation of aqueous extract of *Cucurbita pepo*

C. pepo food material was procured from local market of Shaanxi and authenticated by Professor Wang Wei, The South China Botanical Garden of the Chinese Academy of Sciences. Aqueous extract of *C. pepo* (AECP) was prepared by formerly defined method with slight alterations.[24] The flesh of *C. pepo* shade dried and the powder was extracted with preheated distilled water at 100°C. The ultrasonic water bath assisted extraction for 30 min at 75°C has been achieved (the ratio of *C. pepo* flesh powder to distilled water was 1:20). The final extract was exposed to filtration and then the solvents were disappeared in a rotary evaporator under vacuum at 25°C. The fine powder was stored at −20°C until use.

Animals

Wild-type adult male C57BL/6 mice (n = 80), 10–11 weeks old with body mass 20–25 g, were procured from National Resource Centre of Model Mice (Nanjing, China). Animals were housed in standard approved cages with 5 mice per cage (Sized: 24 cm × 36 cm × 24 cm) and delivered with standard pelleted food and water *ad libitum* in an controlled ventilated vivarium at regulated temperature of 24°C ± 2°C with 50–55 ± 10% humidity, with regular 12 h light-dark cycle. Mice housed were accustomed for about 1 week before the experimental induction and were preserved within the same cages throughout the acclimation and experimental timetable. All procedures containing animals were approved by the animal care committee of the institute and met the guidelines for animal research. Supreme care was provided during each step to minimalize animal suffering and stress. LPS-induced neuroinflammation is recognized in C57BL/6 animal model according to the previous reports.[26]

Cucurbita pepo dosage fixation

C. pepo's effective optimum dosage was secure using an initial study with various dosages of *C. pepo* (aqueous extract) (10, 50, 100, 500, and 1000 mg/kg b.wt), administered via oral route for 7, 15, 30, and 60 days, and then the mice received LPS (1 mg/kg b.w.) intraperitoneally, once for 7 days during the final week of each duration (i.e., 7, 15, 30, and 60 days). It was initiated that *C. pepo* at 100 mg/kg b.w. dose administered for 30 days relapsed the levels of pathophysiological marker enzyme level significantly, i.e., creatine kinase and lactate dehydrogenase to near normalcy without any adverse effects.[25] Hence, we deduced 100 mg/kg b.w. as the optimal dosage for investigating its neuroprotective effect.

Experimental groups and induction schedule

The mice were randomly separated into 4 different groups, with 10 male mice per group (n = 10).

- Group 1: Control group mice received intraperitoneal (i.p.) saline (endotoxin-free injectable solution [saline 200 µl/kg b.w.])
- Group 2: LPS-induced group mice received i.p. LPS (1000 µg/kg b.w.; *Escherichia coli* strain 0111: B4)[27]
- Group 3: LPS induced as in Group 2 (1 mg/kg b.w.) and AECP provided orally (AECP [100 mg/kg b.w.]) for 30 days
- Group 4: Thirty-day intragastric administration of *C. pepo* alone (AECP [100 mg/kg b.w]).

LPS was administered in i.p. injections at doses of 500 µg/kg in saline for period of 1 week and the saline (0.9% NaCl) alone was administered to control mice for each day of testing.

Hematoxylin and eosin staining

This method includes the application of two histological stains, hematoxylin and eosin (H and E). The hematoxylin stains cell nuclei blue and eosin stains the extracellular matrix and cytoplasm pink. This method is regularly used for staining the thin tissue sections of various organs and used for assessment of cellular integrity. The brain tissue segments of 3 µm were fixed in 10% buffered formalin and processed, fixed using paraffin. Freshly dissected tissue sections were placed on clean coated microscopic grade glass slides and stained with H and E.

After staining and fixing, the slides were then visualized under light microscope (Nikon XDS-1B; Nikon, USA).

Assay of antioxidants

All the assays were carried out in cortical tissues obtained from brain of control and experimental rat groups. Total protein amount was enumerated based on Lowry *et al.* method[28] using BSA as control. Activity status of superoxide dismutase (SOD) was determined based on earlier described method.[29] Activities of catalase (CAT) activity was measured, based on the method previously defined method.[30] reduced Glutathione (GSH) was dignified as previously described.[31] glutathione peroxidase (GPX) activity in cortical tissues was determined using previously described method.[32] Formation of thiobarbituric acid reactive substances (TBARS) was employed to assess the lipid peroxidation (LPO) based earlier studies.[33]

Protein extraction from mouse brain

After experimental occupation, the animals were anesthetized using xylazine (50 µl/100 g b.w.) and ketamine (100 µL/100 g b.w). After sedation, the animals were euthanized by decapitation and brain tissues were harvested and the cortex were unglued and kept safely in vials at −80°C and in formalin for histology analyses. The cortical tissues were crushed using PRO-PREP™ protein extraction solution (INtRON Biotechnology). Tissue homogenates were at spin at 12,000 rpm at 4°C for 10–15 min. The clear debris-free supernatants were separated and collected and stored at − 80°C for further assays.

Analysis of gene expression

Gene expressions analysis was performed to assess its mRNA level with the standard real-time (RT)-PCR method.[5] Tissue RNA was isolated using reagent obtained from Sigma Chemical Company as per manufacturer's methods. Reverse transcription and PCR amplification were performed as per protocol. Gene-specific oligonucleotide primers intended for mouse genes were ordered and obtained commercially from vendors: neuronal nitric oxide synthase [nNOS] – sense, 5'-CCTTAGAGAATAAGGAAGGGGGCGGG-3' and antisense, 5'-GGGCCGATCATTGACGGCGAGAATGATG-3' (400-bp size);[33] endothelial nitric oxide synthase (eNOS) – sense, 5' GGGCTCCCTCCTTCCGGCTGC-3' and antisense, 5-'GGATCCCTGGA-AAAG-GCG-3' (260-bp fSize);[34] and inducible nitric oxide synthase (iNOS) – sense, 5'-GCCTCATGCCATTGAGTTCATCAACC-3' and antisense, 5'-GAGCTGTG-AATTCCAGAGCCTGAAG-3 (370-bp size).[5] PCR amplification was performed using standardized programs. After gene amplifications, the samples were cooled to 4°C. About 10–15 of amplified product was loaded onto each individually with 5 µL of sample buffer and separated electrophoretically at 70 V using 2% gel and the amplified products as bands were observed and documented using ethidium bromide. β-actin was used as internal reference control and expression levels of nNOS, eNOS, and iNOS was determined densitometrically.

ELISA

Protein expression for TNF-α, IL-1β, and IL-6 was determined using commercially obtainable ELISA (purchased from Biosource, Invitrogen). Cortex tissues collected from whole brain were then subjected to homogenization by using radioimmunoprecipitation assay lysis buffer (50 mmol/L Tris–HCl (pH 6.8), 150 mmol/L NaCl (sodium chloride), 5 mmol/L EDTA, 0.5% sodium deoxycholate, 0.5% NP-40) with mixture of protease & phosphatase inhibitors (Applygen, Beijing, China) were also added with homogenization buffer on ice-cold conditions. Total protein was then quantified by standard Bicinchoninic Acid (BCA) Protein Assay Kit. From each groups of tissue samples, 5 µL of extracted protein was employed for detection of respective protein expression quantification. Absorbance in spectrophotometer at a wavelength of 450 nm was employed for protein quantification. The amount of TNF-α, IL-1β, and IL-6 was calculated and presented as pg/mg protein.

Immunohistochemistry

Mice brain cortical tissue sections were engrossed in 4% cold buffered formaldehyde and were cut into thin pieces (15 µm). Before incubation with antibodies, the sections were deparaffinized, rehydrated and antigen recovery was done in citrate buffer (10 mM, pH 6.0), quenched for endogenous peroxidase activity (using 3% H_2O_2 in methanol) and blocking was done using tris buffered saline–Tween 20 (TBS-T20) encompassing 1% BSA for 1 h. To the tissue sections coated on glass surface anti-rabbit Ionized calcium-binding adaptor molecule-1 (Iba-1) (1:2000), rabbit polyclonal antibody was added, incubated overnight (O/N) at 4°C in humidified chamber, and washed (thrice) with TBS-T for 5 min. To the tissue sections, secondary antibody (1: 2500) (horseradish peroxidase-conjugated) was added and stained at RT for 2 h. The sections were then stained using activated DAB for 5 min in a dark room under blue light. Development of brown color dots signifies the target protein expression and the number of dots regarded in blinded fashion by three observers independently. Finally, the total number of completely stained cells showing the protein expressions was counted and shown in graphs.

Statistical analysis

SPSS (SPSS Inc., Chicago) and GraphPad Prism (8.0.2) software (Graphpad Software, La Jolla, CA) were used for statistical analyses. Data were expressed as mean ± standard error of mean. All the experiments included more than two groups; using *post hoc* Tukey test, the significance of differences between groups was calculated.

RESULTS

Aqueous extract of *Cucurbita pepo* restores cell morphology

Figure 1 displays the status of cellular morphology of control and experimental groups of mice. Histological checkup was used to assess the morphology of cellular phenotypes which will be an index of physiological and pathophysiological events. Here, LPS-administered mice disclosed intense darkly stained nuclei with swollen cells denoting cellular atrophy and cell damage. Control cells displayed normalcy in its phenotype. AECP-treated mice indicated condensed cell atrophy and significant ($P < 0.05$) reduction in intense darkly stained nuclei compared to LPS administered mice. Mice received AECP without LPS intoxication consider as positive control and brain tissue histology exhibited normal cell morphology similar to normal control (NC) group. This data demonstrates AECP concentration used in this study is non-toxic to normal cells.

Aqueous extract of *Cucurbita pepo* ameliorate enzymatic and non-enzymatic antioxidant level

The LPS-established mice exhibited distinct level of decrease ($P < 0.05$) in both enzymatic and non-enzymatic antioxidants SOD and CAT, as well as GSH and GPx, respectively. In divergence, the level of LPO (TBARS) and nitrite [Table 1] was evidently amplified when compared to the control group. This detected instability was returned to near normal ($P < 0.05$) in the LPS + AECP-treated mice group. I.p. administration of saline 200 µL/kg b.w. and oral administration of AECP 100 mg/kg b.w. to I and

IV groups of animals do not displayed substantial variations in the level of antioxidants, both enzymatic and non-enzymatic.

Effect of aqueous extract of *Cucurbita pepo* on pro-inflammatory cytokine level

Intra-peritoneal administration of LPS to mice initiates the inflammatory events in brain tissue which activates proinflammatory cytokines such as TNF-α, IL-6 and IL-1β. The level of inflammatory cytokines in brain tissue homogenates is accessible in Figure 2. AECP administration in LPS-treated mice abridged the elevation of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β in brain tissue to near normal levels. Quantity of inflammatory molecules raised substantially ($P < 0.05$) than normal control group of mice. On the other hand, AECP (100 mg/kg b.w.) and Normal control (NC) group of mice does not exposed variation in the level of inflammatory cytokines in brain tissue homogenates. These data showed that downregulation of inflammatory cytokines is linked with AECP administration.

Effect of aqueous extract of *Cucurbita pepo* on inflammatory mediators nitric oxide synthase isoforms

To further discover the mechanism of AECP on inflammatory events, the mRNA expressions of isoforms of NO synthase were studied by RT-PCR. Experimental data presented that i.p administration of LPS suggestively ($P < 0.05$) upregulated the expression of nNOS, eNOS, and iNOS mRNA compared to the control groups [Figure 3]. Moreover, compared with mice received LPS, the AECP administration

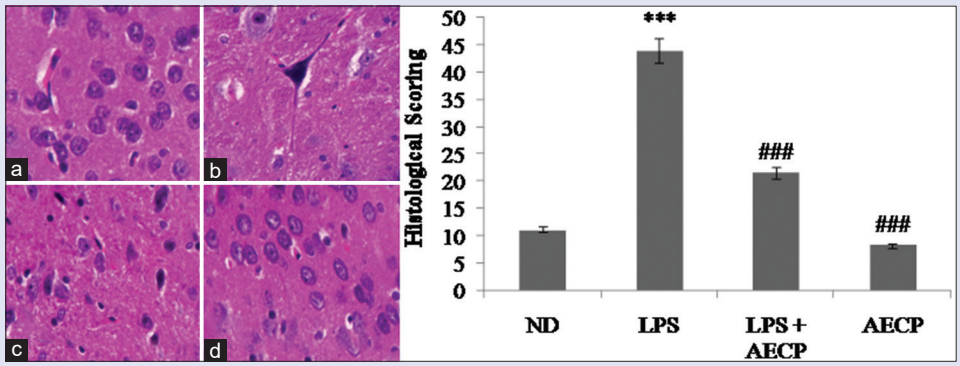

Figure 1: The effect of aqueous extract of *Cucurbita pepo* on the status of brain parenchymal histology performed in brain cortical tissue slices (×20). (a) In control group showing normal intact cell morphology. (b) Strong intense darkly stained nuclei showing swollen neurons and glial cell bodies. (c) Mice received lipopolysaccharide + aqueous extract of *Cucurbita pepo* showed significantly reduced darkly stained and swollen cells compared to inducer group. (d) Group of mice that received aqueous extract of *Cucurbita pepo* alone almost similar to normal control group. Statistically significant ($P > 0.05$) results were presented. Values compared a with b. b with c and d. ***Significantly different from Group I(ND) ($P<0.05$). ###Significantly different from Group II (LPS) ($P<0.05$)

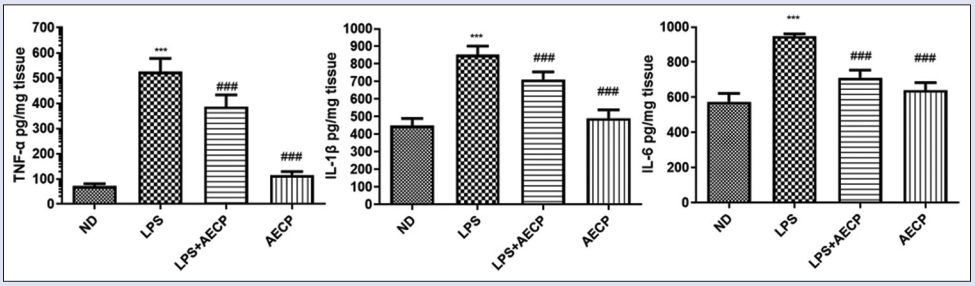

Figure 2: Group I: ND (saline 200 µl/kg bw); Group II: Lipopolysaccharide (1 mg/kg bw); Group III: lipopolysaccharide (1 mg/kg bw) + aqueous extract of *Cucurbita pepo* (100 mg/kg bw); Group IV: Aqueous extract of *Cucurbita pepo* (100 mg/kg bw). Values are expressed in terms of mean ± standard error of mean (6 numbers). ***Significantly different from Group I (ND) ($P < 0.05$). ###Significantly different from Group II (lipopolysaccharide) ($P < 0.05$)

Table 1: Levels of antioxidant enzymes (enzymic and non-enzymatic)

Group	CAT (µmol/ mg protein)	SOD (percentage inhibition/mg/protein)	GPx (µg/min/ mg protein)	GSH (mmol/g of tissue)	TBARS (nM/min/ mg protein)	Nitrite (µM/ml)
I	23.81±1.10	28.75±1.62	6.201±0.138	15.31±0.313	5.32±0.83	9.60±0.28
II	9.60±0.16#	12.23±0.64#	4.273±0.086#	8.04±0.205#	26.29±0.96#	41.25±0.54#
III	16.92±0.47*	21.52±1.74*	5.396±0.132*	17.98±0.428*	12.29±0.45*	17.47±0.39*
IV	22.12±0.82*	26.33±0.82*	5.212±0.28*	13.96±0.416*	6.09±1.01*	10.35±0.66*

*Significant difference from the LPS group ($P<0.05$), #Significant difference from the control group ($P<0.05$). Group I: Control (saline 200 µl/kg bw); Group II: LPS (1 mg/kg bw); Group III: LPS (1mg/kg bw) + AECP (100 mg/kg bw); Group IV: AECP (100 mg/kg bw). Values are expressed as mean±SEM (6 numbers). CAT: Catalase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; GSH: Reduced glutathione; TBARS: Thiobarbituric acid reactive substances; LPS: Lipopolysaccharide; AECP: Aqueous extract of *Cucurbita pepo*; SEM: Standard error of mean

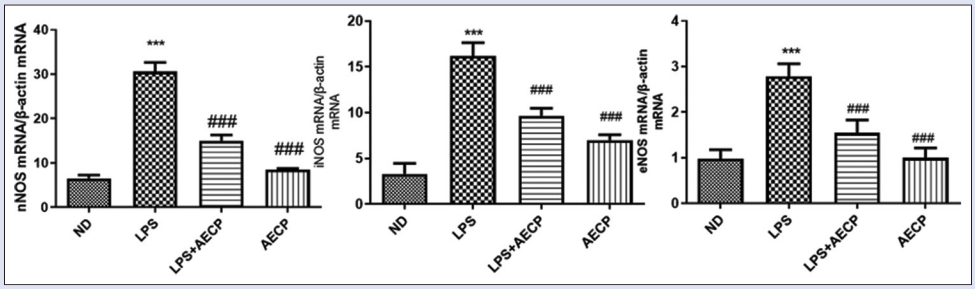

Figure 3: Group I: ND (saline 200 µl/kg bw); Group II: Lipopolysaccharide (1 mg/kg bw); Group III: lipopolysaccharide (1 mg/kg bw) + aqueous extract of *Cucurbita pepo* (100 mg/kg bw); Group IV: Aqueous extract of *Cucurbita pepo* (100 mg/kg bw). Values are expressed in terms of mean ± standard error of mean (6 numbers). ***Significantly different from Group I (ND) ($P < 0.05$). ###Significantly different from Group II (lipopolysaccharide) ($P < 0.05$)

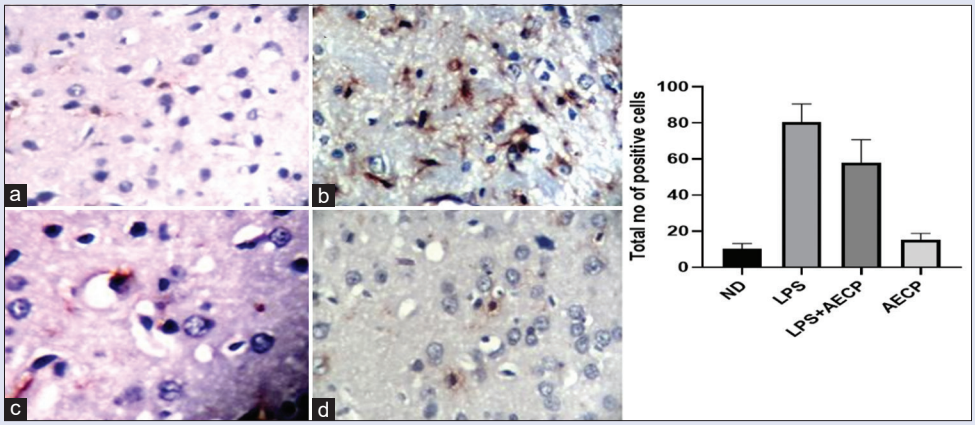

Figure 4: The effect of aqueous extract of *Cucurbita pepo* on the expression of ionized calcium-binding adaptor molecule-1 immunohistochemical staining was performed in brain cortical tissue slices (×20). Positive cells are observed as black/brown dots. (a) In control group, ionized calcium-binding adaptor molecule-1 expression was insignificant. (b) Strong ionized calcium-binding adaptor molecule-1 immunopositivity in lipopolysaccharide-induced toxicity in mice brain. (c) Mice that received lipopolysaccharide + aqueous extract of *Cucurbita pepo* showed 25%–40% less immunopositive cells compared to inducer group. (d) Group of mice that received aqueous extract of *Cucurbita pepo* alone almost similar to normal control group. Statistically significant ($P > 0.05$) results were presented. Values compared a with b. b with c and d

meaningfully deteriorated or condensed the expression level of these inflammatory mediator marker genes. In addition, the administration of AECP alone does not disturb the inflammatory mediator genes and is found to be comparable to control groups of mice.

Effect of aqueous extract of *Cucurbita pepo* on ionized calcium-binding adaptor molecule-1 expression

Figure 4 displays the levels of gliosis (a inflammatory condition) marker expression Iba-1 expression in control and experimental mice groups. LPS-persuaded mice exhibited meaningfully ($P < 0.05$) augmented expression of Iba-1 expression compared to control. AECP-administered groups of mice displayed a significant ($P < 0.05$) reduction in the levels of Iba-1 expression compared to LPS-challenged mice. Moreover, AECP-administered groups and control groups of mice did not exhibit any marked expression for Iba-1 proteins.

DISCUSSION

Although there are large number of synthetic drugs in the market for therapeutic treatment of various ailments of CNS including neurodegenerative and psychiatric illness, search for plant resultant natural agents with potential advantages is still ongoing.[34] With that interest, in this study, we envisioned to examine the neuroprotective potential of AECP against LPS-challenged neuroinflammation in an experimental mice model. *C. pepo* is an annual, monoecious climber seen in Mexico and also cultivated globally for its edible fruits. C. Pepo well known for its phenolic content attributed antioxidant potential. Traditionally *C. pepo* used to nephritis, tuberculosis, internal worms and parasites associated ailments.[35] Particularly, its beneficial effects against anti-inflammatory and anti-microbial activity have been clarified elsewhere till date.[36]

Oxidative stress-induced neuroinflammation includes changed intracellular signals leading to activation of pro-inflammatory genes whose activation ultimately favors neurodegeneration.[37,38] In this study, LPS administration to C57BL/6 mice resulted in deficient antioxidant defense (both enzymic and non-enzymic), main typical feature of oxidative stress as a result of free radical generation during LPS challenge.[39] Indeed, oxidative stress is known to mediate disrupted cellular morphology and expression of pro-inflammatory genes (TNF-α, IL-1β, IL-6, and NO). Moreover, the expression of the above stated inflammatory genes is further escorted by onset of deleterious effects of NO derived from expression of isoforms of NO synthases (eNOS, nNOS, and iNOS).[40,41] In this study, LPS administration-induced elevated expressions of TNF-α, IL-1β, and IL-6 and isoforms of NO synthases (eNOS, nNOS, and iNOS), respectively, represent that the oxidative niche is further governed by elevated NO generation in brain cortical tissues of LPS-challenged mice leading to neuroinflammation.

AECP administration to LPS-challenged mice occasioned in attenuation of the abnormalities in the brain parenchymal histology as evident from swollen neurons and swollen glial cells. Furthermore, AECP restored the inflammatory niche and accompanied NO production to normalcy. Moreover, our results were found to verify with similar documented report.[42] This demonstration AECP has the potential to restore the changes in redox balance state to its homeostasis. Hence, the beneficial effects of AECP as antioxidative and anti-inflammatory agents against neuroinflammation are clarified in this study.

Further in order to clarify the anti-inflammatory effect of AECP against specific cellular phenotypes, effect of AECP against activation of microglial cells during LPS intoxication was assessed. Iba-1 is a cytoplasmic protein, and numerous studies have described its activation to associate with microglial activation and neuroinflammation.[43,44] In this study, the total immunoreactivity area of Iba-1 was augmented in the cortical tissues of LPS-induced C57BL/6 mice compared to cortical regions of control mice. AECP treatment diminished the hyperactivity of microglial cells thereby reduce scar regions in brain tissue histology study. This in turn suggests the anti-inflammatory potential of AECP against LPS-induced neuroinflammation. Results of immunoreactivity study of Iba-1 in AECP treated mice agreed with dietary flavonoid compound fisetin mediated controlled expression of Iba-1.[5]

Recently, quantitative analysis of bioactive compounds on *C. pepo* substantiates the contents of various phytoconstituents; it comprises carotenoids, tocopherols, phenolic acids, flavonols, mineral compounds, and vitamins. Further, studies on quantitative analysis of phytochemicals explored that minerals are most abundant than any of other compounds. In the case of phenolic compounds, it has been quantified nine of mostly stated bioactive compounds in previous studies about *C. pepo*, where caffeic acid is most rich (72 mg/100 g dm) and ferulic acid is the second most copious (16 mg/100 g dm) among phenolic compounds. Whereas, it was found that rutin (12 mg/100 g dm) is the most abundant falvonol group of compound than six other quantified falvonols.[45] Moreover, number of studies often validate the neuroprotective role of phenolic acids such as caffeic acid and ferulic acids by using various *in vitro* models. Meanwhile, studies demonstrate the neuroprotective efficiency of flavonols comprise rutin and kaempferol. Further, studies imagined that identified antioxidant potential of these compounds being the mechanistic factor behind the role of neuroprotection.[46] In summary, this study showed the beneficial antioxidant and anti-inflammatory effect of AECP against LPS-induced neuroinflammation.

CONCLUSION

AECP probably via its ability to scavenge free radicals derived from LPS induction provides antioxidant defense and also deters neuroinflammation and its accompanied NO generation. Hence, we powerfully propose AECP for its therapeutic efficacy as a neuroprotective agent in clinical use. However, this study has boundaries on the mechanistic role of AECP for its neuroprotective function and will be recognized in upcoming studies.

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Conflicts of interest

There are no conflicts of interest.

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