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RESEARCH

Chrysophyllum albidum (African star apple) fruit-supplemented diet enhances cognitive functions and attenuates lipopolysaccharide-induced memory impairment, oxidative stress, and release of proinflammatory cytokines

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Abstract

Purpose Fruit-based supplement has an important role in protecting the brain against oxido-inflammatory stress. *Chrysophyllum albidum* fruit contained several phytonutrients that possess antioxidants and anti-inflammatory properties. Hence, this study investigated the effect of *C. albidum* fruit supplemented diet (CAFD) on cognitive functions and lipopolysaccharide (LPS)-induced memory impairment and oxido-inflammatory response in mice.

Methods Mice were randomized into two experiments. Experiment 1 with naïve mice contained four groups ($n = 6$) while experiment 2 with LPS contains five groups ($n = 6$). Mice in experiments 1 and 2 were fed on CAFD (5%, 10%, and 20%) in naïve (6 weeks) and LPS (250 μg/kg, i.p.) in the 7th week, respectively. Cognitive performance was tested using Y-maze test (YMT) and novel object recognition test (NORT) in the naïve and LPS mice. Brain samples were obtained for determination of oxido-inflammatory parameters and acetylcholinesterase activity.

Results The CAFD significantly enhanced cognitive performance in the YMT and NORT in naïve and LPS mice, as evidenced by increased % alternation and discrimination index, respectively. CAFD supplementation significantly reduced acetylcholinesterase enzyme activity while it attenuated depletion of reduced glutathione and catalase activities in brains of naive and LPS-treated animals. The CAFD significantly reduced LPS-induced increased malondialdehyde levels in mice brains. CAFD supplementation significantly attenuated LPS-induced pro-inflammatory cytokines (IL-6, TNF-α) in mice brains.

Conclusion *Chrysophyllum albidum* fruit supplementation in diet enhances memory function and prevents cognitive deficits induced by LPS via mechanisms associated with inhibition of oxidative stress-related processes, acetylcholinesterase activity, and pro-inflammatory mediators.

Keywords *Chrysophyllum albidum* · Memory · Lipopolysaccharide · Neuroinflammation

Introduction

The high prevalence of micronutrient deficiencies in developing countries has been attributed to the inadequacy in knowledge of the nutritional value of fruits and vegetables, as well as their low consumption, despite their availability in these countries [1]. The health-benefiting and health-promoting properties of fruits are due to their richness in micronutrients and phytochemicals required for the growth, development, and optimal functioning of the human body [1]. As part of a healthy diet low in fat, sugars, and sodium, the World Health Organization (WHO) suggests consuming more than 400 g of fruits and vegetables per day. Frequent consumption of fruits and vegetables has been associated with lowered vulnerability risk factor for non-communicable diseases (NCDs) such as Alzheimer's disease, Parkinson's disease, depression, autoimmune diseases, diabetes, coronary heart disease, cancer, stroke, osteoarthritis, osteoporosis, and

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others [2] and possibly delayed onset of age-related disorders. Recent findings showed that NCDs are the leading cause of death globally. Expanded body of epidemiological studies have shown that NCDs kill approximately 41 million people each year, accounting for about 85% of all premature deaths globally [3]. The 2030 Agenda for Sustainable Development recognizes NCDs as a major challenge for sustainable development particularly in developing countries [3].

Indeed, several epidemiological reports have shown the involvement of systemic bacterial, parasitic, or viral infections including influenza, *Toxoplasma gondii*, and COVID-19 in the vulnerability and pathogenesis of some neurodegenerative diseases in developing countries [3–6]. Emerging evidence shows that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogenic agent for coronavirus disease 2019 (COVID-19), is a neuroinvasive virus capable of causing neurodegeneration and neuropsychiatric disease via cytokine storm [7, 8]. These evidences have led to the design of animal models involving exposures to infectious compounds to mimic certain features of some neurodegenerative disease [6, 9, 10]. Accordingly, lipololysaccharide (LPS) is a widely used endotoxin of gram-negative bacteria popularly used to simulate behavioral and neuroinflammation and oxidative biochemical changes that characterized neurodegenerative pathology including conditions of cognitive dysfunction [10]. Neuroinflammation and oxidative stress has distinct features that are shared in aging and in neurodegenerative diseases such as stroke and Alzheimer's disease (AD) [11]. The activation of the immune system in response to an infectious or bacterial endotoxin, LPS produces profound neurophysiological, neuroendocrine, and behavioral changes [12]. Therefore, the injection of LPS promotes the production and release of several cytokines [13]. In response to these cytokines, several reactive oxygen species are produced from cells such as neutrophils and other phagocytic cells, creating a status of vicious oxidative stress [12]. Although acute neuroinflammation plays a protective role, chronic neuroinflammation is frequently considered detrimental and damaging to nervous tissues [14]. LPS-induced neuroinflammation has been repeatedly linked to microglia stress and modulation of broad spectrum of cellular responses partly due to repeated exposure to infectious agents [6]. Thus, LPS serves as neuroinflammatory tool for the screening of compounds with neuroprotective property [15].

Considering the fact that the global incidence of noncommunicable neurological disorders are increasing at an alarming rate, there is a global move toward the development of cost-effective natural neuroprotective phytochemicals [3, 16, 17]. As part of this agenda, research institutions and Government policy on health are committed to develop ambitious national responses, by 2030, to reduce by one-third premature mortality from NCDs through prevention and treatment, which include dietary modification [3]. Indeed, a nutritional approach could be a potential strategy to prevent or slow the progression of some NCDs especially neurodegenerative diseases, as experimental and clinical studies have reported significant decrease in risk factor for neurological NCDs and phytochemical supplementations [16, 17]. Phytochemicals are required for the maintenance of cell viability and protection of neural cells from neuroinflammation and oxidative stress associated with aging and brain diseases [9, 17]. Although the mechanisms of nutritional impact on the brain are complex, fruits and its juice impart beneficial effect on brain functioning. In recent years, various groups of researchers have performed food-based intervention studies to reveal the effect of dietary factors on certain mechanisms that take care of mental activity and the potential of phytochemical-rich foods to prevent age-related neurodegeneration and cognitive decline [18, 19]. In particular, evidence suggests that foods rich in flavonoids possess the greatest potential to act on cognitive processes; notably, via selective modulation of protein kinase and lipid kinase signaling cascades, which regulate transcription factors and gene expression involved in both synaptic plasticity and cerebrovascular blood flow [20, 21]. Indeed, increased hippocampalneurogenesis by diet has been linked repeatedly to improved cognition performance, brain plasticity, and mental health [19, 22]. Also, there has been expanding body of evidence showing strong connections between poor dieting, cellular aging, and cognitive impairment failure [23].

Chrysophyllum albidum (Linn), also known as African star apple, belongs to the family *Sapotaceae*. It is primarily a forest tree species with its natural occurrences in diverse ecozones in Nigeria, Uganda, and Niger Republic [24]. In Nigeria, *C. albidum* is known as "agbalumo" in South Western Nigeria and "udara" in South Eastern Nigeria. The fruit is seasonal with immense economic potential, especially following the report that jams obtained from the fruit-pulp could compete with raspberry jams and jellies, while the oil from the seed has been used for diverse medicinal purposes [2]. Its rich sources of natural antioxidants have been established to promote health by acting against oxidative stress-related diseases such as diabetes, cancer, and coronary heart diseases [25]. The fruit-pulp has been reported to contain significant amount of ascorbic acid, vitamins, iron, food flavors, fat, carbohydrate and mineral elements [2]. The fruit-peel has been shown to be a rich source of fiber and mineral while the seed shell pericarp has been reported to be good source of carbohydrate and minerals [26]. The fruits are not only consumed fresh but also used to produce stewed fruit, marmalade, syrup, and several types of soft drinks [27]. Its high pectin content [28] is also suggestive of its vast medicinal benefits, which includes plasma cholesterol level reduction [29], as well as its detoxifying action and effectiveness in diarrhea therapy. However, there is a significant gap in knowledge of the neuroprotective property of *C. albidum* in disease conditions related to oxidative stress and neuroinflammation in

experimental animals. As part of our ongoing studies of the neuropharmacological activity of *C. albidum*, we therefore hypothesized that (i) supplementation of *C. albidum* fruit supplemented diet (CAFD) would improve memory performance in naïve and LPS-treated mice, (ii) prolonged CAFD supplementation could prevent oxidative and nitrergic alterations in naïve and LPS-treated mice, and (iii) repeated CAFD supplementation would attenuate pro-inflammatory proteins release in naïve and LPS-exposed mice.

Materials and methods

Chemicals and reagent

Lipopolysaccharide-LPS (*Escherichia coli* serotype, 055:B5), acetylthiocholine, Ellman Reagent [5′, 5′-dithiobis-(2-nitrobenzoate) DTNB] and thiobarbituric acid (TBA) were obtained from Sigma-Aldrich, St. Louis, USA. Trichloroacetic acid (TCA) was obtained from Burgoyne Burbidges & Co., Mumbai, India. Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) ELISA kits were purchased from BioLegend (USA).

Sample collection and preparation

Fresh and ripe *Chrysophyllum albidum* fruit samples obtained from Bodija market, Ibadan North LGA of Oyo State were identified and authenticated at the herbarium of Forestry Research Institute of Nigeria (FRIN) Ibadan, and herbarium voucher number was issued as FHI 110475. The fruit samples were thoroughly washed with clean water. The seeds were removed and the pulp and peel of the fruits cut into small pieces. Then, the pieces oven dried at 50 °C for 10 days. It was then pulverized to power using a mechanical grinder.

Feed supplementation process

Diets were made from commercially formulated feed (Top Feeds, Ibadan, Nigeria) containing crude protein 23.00%, fat/oil 6.00%, crude fiber 5.00%, calcium 1.00%, available phosphorus 0.40%, lysine 1.20%, methionine 0.50%, salt (min) 0.30%, and metabolizable energy (min) 2900 kcal/kg. The CAFD was formulated to contain 5%, 10%, and 20% CAFD. Five percent CAFD supplemented diet had 5 g of the CAFD sample mixed with 95 g of standard diet while 10 g and 20 g of the sample was mixed with 90 g and 80 g of the standard diet for 10% and 20% supplementation, respectively. Supplemented and non-supplemented diets were homogenized using a mixer and made into pellets.

Experimental animals

A total of seventy-two male Swiss mice (20–23 g) used for the study were obtained from the Central Animal House, University of Ibadan. The experimental animals were weighed, marked, and randomly divided into five groups. They were housed in plastic cages with wood shavings as bedding, at room temperature and normal relative humidity. They had free access to food pellets and water ad libitum. The animals were acclimatized for 1 week and kept under 12 h light/dark cycles throughout the experiments. Body weight was taken weekly. Animal care and use procedure for the experiment were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/18/0054).

Evaluation of the memory enhancing and neuroprotective effects of CAFD in naive and LPS-induced neuroinflammation in mice

Mice ($n = 12$) were fed with CAFD and non-supplemented diets in two experimental protocols. Experiment 1 with naïve mice contained four groups ($n = 6$) while experiment 2 with LPS contained five groups ($n = 6$). Mice in experiments 1 and 2 were fed on CAFD (5%, 10%, and 20%) in naïve (6 weeks) and LPS (7 weeks), respectively. In experiment 1, group 1 mice were fed with basal diet, while groups 2–4 were fed with CAFD (5%, 10%, and 20%) for 6 weeks. In experiment 2, group 1 received non-supplemented diet (basal diet) and served as normal control, group 2 received non-supplemented diet (basal diet), and groups 3–5 had 5%, 10%, and 20% CAFD supplemented diet, respectively, for 7 weeks. Mice in groups 2–5 were intraperitoneally treated with lipopolysaccharide LPS (250 μg/kg) [10] for 7 days during the 7th week.

Neurobehavioral assessment

Mice were tested for behavioral phenotypes 24 h after the last administration of LPS on day 49 (spatial and non-spatial cognitive function, social recognition memory, locomotion). Each of the apparatus used for the behavioral tests were cleaned with 70% ethanol before the first test and also in between tests.

Y-maze test

Spontaneous alternation behavior in the Y-maze was assessed as a measure of spatial memory function. The Y-maze test was used to assess the effect of CAFD supplementation on working memory in mice. Mice were placed individually in the Y-maze and allowed to explore all the three arms freely for 5 min. The parameters assessed were number of arm entries (i.e., A, B, C or B, C, A, etc.) and alternations. An entry was

scored when the four paws of the animals were completely in the arm of the Y-maze. The percentage alternation, which indicated memory performance, was calculated [30] by dividing the total number of alternations by the total number of arm entries, minus 2 and multiplied by 100, i.e., [successive triplet sets/(total number of arm entries − 2)] × 100.

The novel object recognition task

The effect of CAFD supplementation on non-spatial memory performance was also assessed using the NORT in an open-field chamber (60 cm × 50 cm × 40 cm) as previously described [31]. The discriminated objects (A, B, and C) were identically sized (4.5 cm diameter and 11.5 cm height) cylindrical bottles. Objects A and B were white, whereas object C had a black and white pattern. The NORT consists of two phases: the trial phase and the test phase. The animals were acclimatized to the experimental condition for a period of 5 min. The trial phase was carried out by placing each mouse in the middle of two identical objects (A and B) on opposite sides of the open-field chamber for 5 min. Thereafter, the animals were returned to their home cages for an interval of 4 h. In the test phase, object B was replaced with object C, which was novel to the mice and different from either object A or B. Mice were then left to explore objects A and C for a period of 5 min. The apparatus was cleaned after each test, and the time spent in exploring each of the objects was recorded in both phases. The discrimination index, which was used as a measure of non-spatial memory function, was calculated as the difference in time exploring the novel and familiar object divided by the total amount of time spent with both objects.

Social recognition memory

This test is based on the observation that a familiar mouse tends to explore the unfamiliar counterpart more at first exposure than at second exposure (fixed inter-trial intervals), was carried out as recently described [9]. The decrement in exploration time between the first and second exposure is therefore used as an indication of social recognition memory. The test, which consists of two exposure sessions: the pretest (training; first exposure) session and test (second exposure) session was conducted in an observation chamber (29 × 18 × 12 cm). Prior to the first exposure, test mice (familiar mice) and unfamiliar (non-experimental social interacting mice) mice were brought to the observation room and were left to acclimatized to the new environment for 1 h. The training session was carried out by placing each experimental mouse into the observation chamber and allowed to habituate to the test environment for 15 min. Thereafter, the non-experimental (unfamiliar) mouse was placed into the same observation chamber with the experimental mouse. The duration of social investigatory behaviors, which include direct contact or sniffing, nosing, grooming, pawing or inspection of body parts of the unfamiliar (non-experimental) mouse was scored by a trained observer for a period of 5 min. After the first exposure (training session), the unfamiliar mice were returned to their home cages for an interval of 30 min (for short-term memory) or 24 h (for long-term memory consolidation of social recognition memory). The re-exposure (test sessions) were carried out 30 min after the pretest session between the same experimental and non-experimental counterpart. The time spent by the experimental mice in exploration of unfamiliar mice at inter-trial intervals was measured. The social recognition memory was defined as a decrement in the exploration time between the first and second (fixed inter-trial intervals) exposures for the short- and long-term social recognition memories [9]. The test box was also cleaned with 70% ethanol to avoid instinctive odorant cues from previous animal to the next.

Performance of mice in the activity cage (OFT)

The activity cage measures spontaneous coordinate activity, meaning movement in either the horizontal or vertical plane of experimental animals within a specified amount of time. The locomotion function of the mice was evaluated using the activity cage preoperatively after feeding mice with *C. albidum* fruit-supplemented diet [32].

Biochemical assays

After the behavioral tests, the animals were sacrificed under ether anesthesia and the brains were rapidly removed. Thereafter, the whole brain was weighed and homogenized with 10% *w*/*v* phosphate buffer (0.1 M, pH 7.4). The brain tissue homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatants were stored at − 20 °C for determination of acetyl-cholinesterase activity, reduced glutathione, catalase, malondialdehyde, nitrite, and pro-inflammatory cytokines. Reduced glutathione (GSH) was estimated using DTNB as described by Jollow et al. [33]. The extent of lipid peroxidation was determined by quantifying the level of malondialdehyde (MDA) [34].The activity of catalase (CAT) was determined according to the method previously described by Goth [35].

Determination of AChE activity in mice brain

Aliquots of supernatants of the individual mouse brain of the various treatment groups were taken and used to measure acetylcholinesterase (AChE) activity, a marker for cholinergic neurotransmission. Briefly, AChE activity in the homogenate was measured by adding 1.3 mL of phosphate buffer (0.1 M, pH 7.4), 0.05 mL of 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB), and 0.1 mL of the homogenate. Then, 0.05 mL of acetylthiocholineiodide was added to the reaction mixture. The

absorbance was read using a spectrophotometer at a wavelength of 412 nm and change in absorbance for 3 min at 1-min interval was recorded. The rate of AChE activity was measured by following the increase of color produced from thiocholine when it reacts with DTNB. The change in absorbance per minute was determined, and the rate of AChE activity was calculated and expressed as μmol/min/mg protein [36].

Estimation of brain nitrite level

Brain nitrite concentration was estimated using Griess reagent, which serves as an indicator of nitric oxide production [37]. One hundred microliter of Greiss reagent (1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% of N-1-naphthyl ethylenediamine dihydrochloride) was added to 100 μL of the supernatant and absorbance was measured at 540 nm [38]. The brain nitrite concentration was estimated from a standard curve obtained from sodium nitrite (0–100 μM).

Detection of TNF-α and IL-6

The concentrations of TNF-α and IL-6 in the supernatants of the brain were determined according to the manufacturer's instructions. TNF-α and IL-6 were measured by specific mouse TNF-α (BioLegend ELISA MAX™ Deluxe kit, USA) and IL-6 (BioLegend ELISA MAX™ Deluxe kit, USA) with sensitivity limit of 4 pg/mL. All the measurements were done at room temperature in accordance with BioLegend instructions using microplate reader with 450 nm filter. The concentrations of TNF-α and IL-6 from the tissues were extrapolated from the standard curve of TNF-α and IL-6 standards included in the assay kits. The levels of TNF-α and IL-6 in the brain were expressed as pg/g tissues.

Statistical analysis

The data were analyzed using GraphPad Prism software version 5.0 and expressed as mean ± SEM. Statistical analysis was done using one-way ANOVA, followed by Newman–Keuls post hoc test. *P* values less than 0.05 were considered statistically significant.

Results

Effects of CAFD on body weight

The effect of feeding mice with CAFD 5, 10, and 20% for 6 weeks on body weight is shown in Fig. 1. There was no significant difference in animals fed with CAFD 5, 10, and 20% when compared to basal diet control, although there was a gradual increase in body weight from week 3 to week 6.

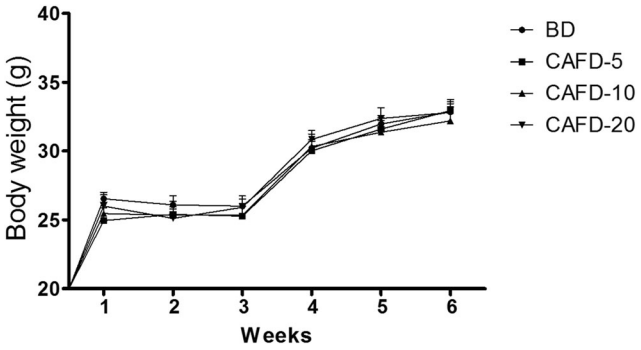

Fig. 1 The trend of the average weights of the mice. Data are expressed as mean ± SEM ($n = 12$). BD basal diet. CAFD *Chrysophyllum albidum* fruit-supplemented diet at 5%, 10%, and 20% supplementation

Chrysophyllum albidum fruit diet enhanced and improved spatial memory performance in naïve and LPS-treated mice

The effect of CAFD supplementation for 6 weeks on spatial memory performance in naive mice and LPS-exposed mice using the Y-maze paradigm is shown in Fig. 2a, b. One-way ANOVA showed that there were significant differences between treatment groups: % correct alternation behavior [$F(3, 25) = 7.322$; $p = 0.0014$]. Post hoc analysis by Newman–Keuls multiple comparison test showed that 20% CAFD fed mice showed significant ($p < 0.05$) increased % correct alternation when compared to basal diet mice control (Fig. 2a). One-way ANOVA revealed that LPS (250 μg/kg, i.p.), administered once daily for 7 days, significantly decreased the % level of alternation behavior in comparison with BD, suggesting memory impairment. However, CAFD 5, 10, and 20% groups did not significantly reverse LPS-induced memory impairment (Fig. 2b).

Chrysophyllum albidum fruit-supplemented diet enhances non-spatial working memory in naïve mice and attenuates LPS-induced non-spatial working memory impairment

The effect of CAFD supplementation for 6 weeks on memory performance in 8-week-old mice is shown in Fig. 3a, b. One-way ANOVA showed that there were significant differences between treatment groups: Discrimination index [$F(3, 30) = 5.939$; $p = 0.0030$]. Post hoc analysis by Newman–Keuls multiple comparison test showed that 5%, 10%, and 20% CAFD fed mice significantly ($p < 0.05$) increased discrimination index when compared to basal diet fed mice (Fig. 3a). Meanwhile, intraperitoneal injection of LPS (250 μg/kg) given once daily for 7 days significantly ($p < 0.05$) decreased the index of recognition memory in comparison with the basal diet group, suggesting impairment of memory function. However, pre-feeding with CAFD 10 and 20% significantly ($p < 0.05$) reversed LPS-induced memory impairment (Fig. 3b).

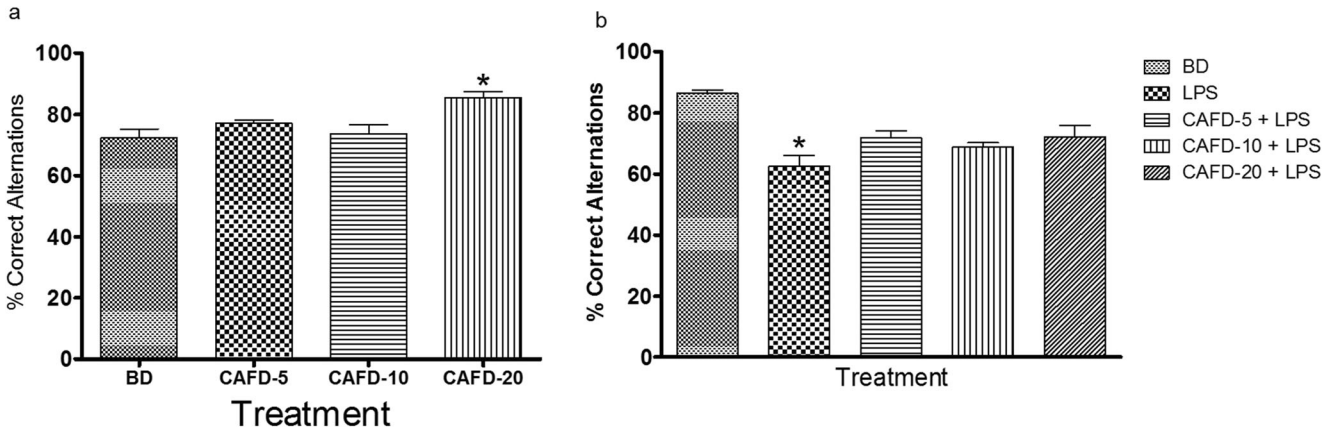

Fig. 2 Effect of *Chrysophyllum albidum* fruit-supplemented diet on naïve mice (**a**) and LPS-induced spatial working memory impairment (**b**) in mice in the Y-maze test. Data are expressed as mean ± SEM ($n = 6$). *$p < 0.05$ vs BD using one-way ANOVA followed by Newman–Keuls post hoc test. BD basal diet. CAFD *Chrysophyllum albidum* fruit-supplemented diet at 5%, 10%, and 20% supplementation

Chrysophyllum albidum fruit-supplemented diet enhances social recognition memory performance in naïve mice

The effect of CAFD supplementation for 6 weeks on social memory performance is shown in Fig. 4. One-way ANOVA showed that there were significant differences between treatment groups: Social memory index [$F(3, 19) = 26.09$; $p = 0.1626$]. Post hoc analysis by Newman–Keuls multiple comparison tests showed that CAFD-fed mice significantly ($p < 0.05$) increased social memory index when compared to basal diet fed mice (Fig. 4).

Effect of *Chrysophyllum albidum* fruit-supplemented diet on horizontal and vertical activities in LPS-treated mice in the activity cage

The effects of CAFD on spontaneous coordinate activity as assessed by the horizontal and vertical activities in the open field test (activity cage) are presented in Fig. 5a, b. LPS exposure caused significant decreased horizontal (Fig. 5a) and vertical (Fig. 5b) activities relative to BD controls. Although CAFD (5–20%) did not reverse the alteration on horizontal movement, CAFD 10% significantly prevented LPS-induced alteration in spontaneous motor activity (Fig. 5a, b).

Chrysophyllum albidum fruit-supplemented diet reduces acetylcholinesterase activity in naïve and LPS-induced increased acetylcholinesterase activity in mice brains

The effect of feeding mice with CAFD 5, 10, and 20% for 6 weeks on the activity of AChE in naïve and LPS-treated mice brains are shown in Fig. 6a, b. One-way ANOVA showed that there were significant differences between treatment groups. Post hoc analysis by Newman–Keuls test revealed that CAFD 10 and 20% significantly ($p < 0.05$) inhibited AChE activity when compared with the basal diet

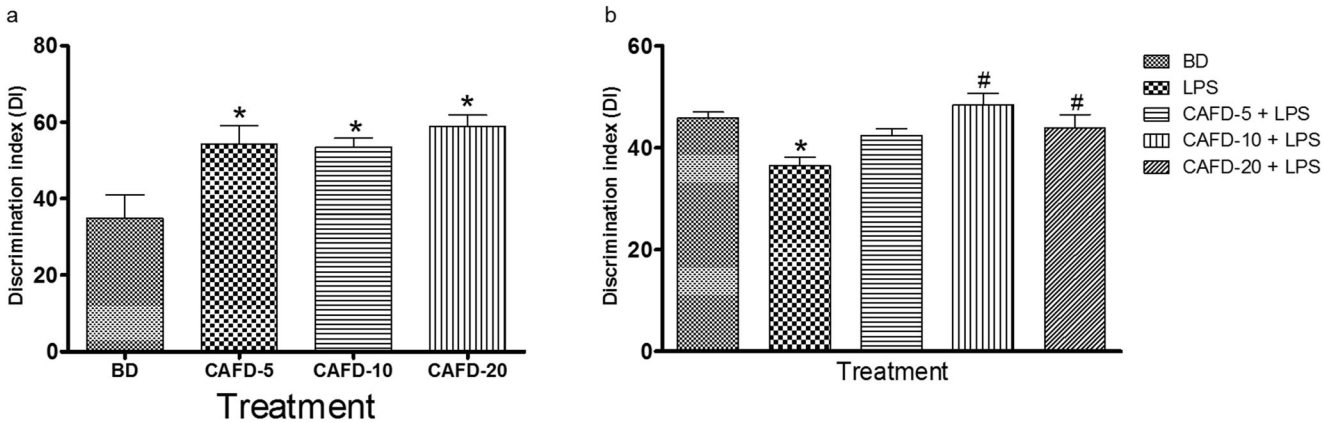

Fig. 3 *Chrysophyllum albidum* fruit-supplemented diet (CAFD) enhances non-spatial working memory in naïve mice (**a**) and attenuates LPS-induced non-spatial working memory impairment (**b**) in mice. Data are expressed as mean ± SEM ($n = 6$). *$p < 0.05$ vs BD; #$p < 0.05$ compared with LPS (one-way ANOVA followed by Newman–Keuls post hoc test). BD basal diet, LPS lipopolysaccharide, CAFD *Chrysophyllum albidum* fruit-supplemented diet at 5%, 10%, and 20% supplementation

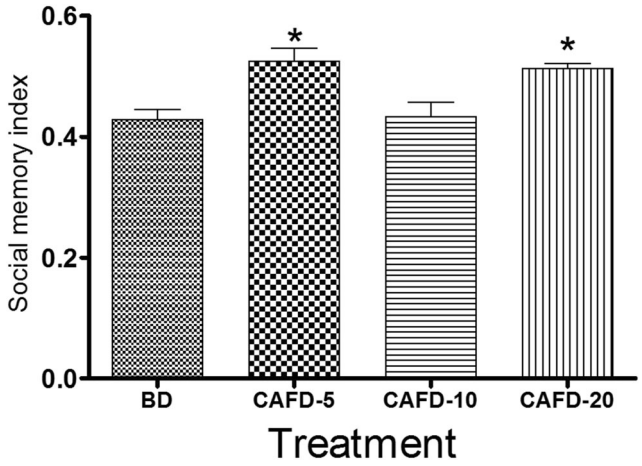

Fig. 4 *Chrysophyllum albidum* fruit-supplemented diet (CAFD) enhances social recognition memory performance in mice. Data are expressed as mean ± SEM ($n = 6$). *$p < 0.05$ vs BD using one-way ANOVA followed by Newman–Keuls post hoc test. BD basal diet, CAFD *Chrysophyllum albidum* fruit-supplemented diet at 5%, 10%, and 20% supplementation

fed mice (Fig. 7a). As shown in Fig.7b, LPS significantly ($p < 0.05$) increased brain AChE activity in comparison with BD in mice. Pre-feeding with CAFD 5, 10, and 20% produced a significant ($p < 0.05$) reduction of AChE activity when compared with the LPS group.

Chrysophyllum albidum fruit-supplemented diet increases antioxidant levels in naïve and LPS-induced altered antioxidant levels in mice brains

Figure 7a and b show the effect of feeding mice with CAFD 5, 10, and 20% for 6 weeks on GSH concentration in naïve and LPS-treated mice brains. One-way ANOVA and post hoc analysis by Newman–Keuls test revealed that CAFD significantly ($p < 0.05$) increased the concentration of glutathione (GSH) in mice brains in comparison with the basal diet group (Fig. 7a). As shown in Fig. 7b, LPS (250 μg/kg, i.p.) caused a profound depletion of brain levels of GSH relative to the BD group ($p < 0.05$). However, CAFD 10 and 20% significantly ($p < 0.05$) attenuated GSH depletion caused by LPS (Fig. 7b).

Further, one-way ANOVA showed that there were significant differences between treatment groups. Consumption of CAFD produced a differential effect on CAT activity. Accordingly, while lower CAFD 5% showed no significant effect on CAT activity, higher percentages (CAFD 10% and 20%) markedly increased CAT activity in mice brain following 6 weeks of continuous consumption of CAFD in comparison with BD group (Fig. 7c). On the other hand, LPS (250 μg/kg, i.p.) was found to significantly ($p < 0.05$) reduce catalase activity relative to basal diet. But, pre-feeding with CAFD 5, 10 and 20% also attenuated the effect of LPS on CAT activity in a significant ($p < 0.05$) manner (Fig. 7d).

Effects of *Chrysophyllum albidum* fruit-supplemented diet on lipid peroxidation levels in naïve and LPS-treated mice brains

Further, one-way ANOVA revealed that there are significant differences between treatment groups. Repeated consumption of CAFD 5, 10, and 20% did not cause any significant alteration on lipid peroxidation as shown by MDA levels in comparison with BD (Fig. 8a). As shown in Fig. 8b, LPS (250 μg/kg, i.p.) produced a significant ($p < 0.05$) increase in lipid peroxidation as indicated by increased MDA concentrations in mice brains in comparison with the basal diet group. However, CAFD 5, 10, and 20% significantly ($p < 0.05$) reduced brain concentrations of MDA caused by LPS indicating antioxidant property.

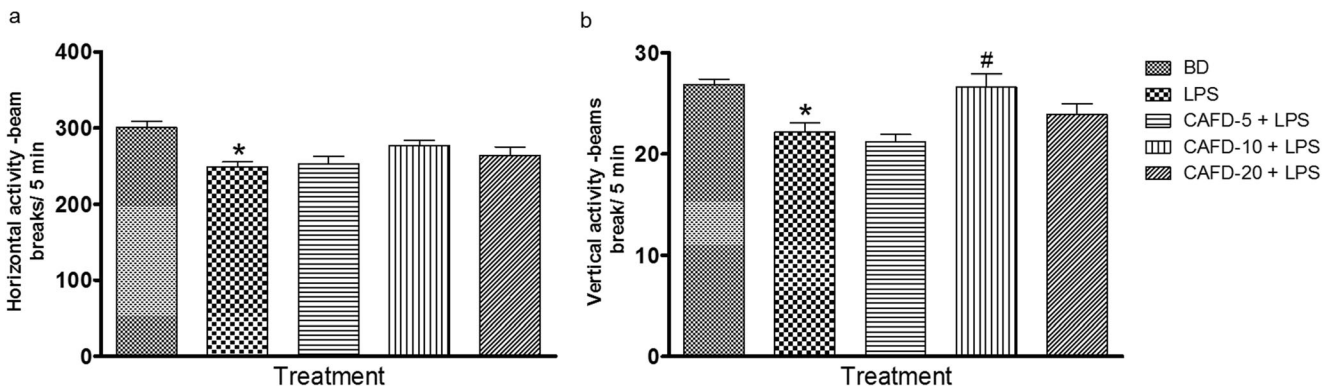

Fig. 5 Effect of *Chrysophyllum albidum* fruit-supplemented diet on horizontal (**a**) and vertical (**b**) activities in LPS-treated mice in the activity cage. Data are expressed as mean ± SEM ($n = 6$). *$p < 0.05$ vs BD; #$p < 0.05$ compared with LPS (one-way ANOVA followed by Newman–Keuls post hoc test). BD basal diet, LPS lipopolysaccharide, CAFD *Chrysophyllum albidum* fruit-supplemented diet at 5%, 10%, and 20% supplementation

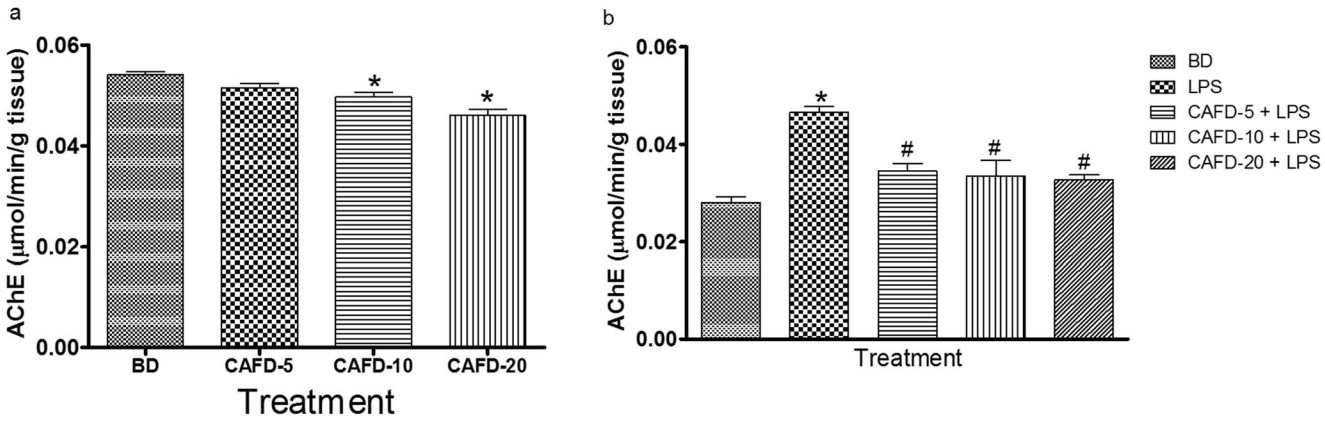

Fig. 6 *Chrysophyllum albidum* fruit-supplemented diet reduces acetylcholinesterase activity in naïve (**a**) and LPS-induced increased acetylcholinesterase activity (**b**) in mice brains. Data are expressed as mean ± SEM ($n = 6$). *$p < 0.05$ vs BD; #$p < 0.05$ compared with LPS (one-way ANOVA followed by Newman–Keuls post hoc test). BD basal diet, LPS lipopolysaccharide, CAFD *Chrysophyllum albidum* fruit-supplemented diet at 5%, 10%, and 20% supplementation

Chrysophyllum albidum fruit-supplemented diet decreases brains levels of proinflammatory cytokines in mice brains submitted to lipopolysaccharide

Figure 9a and b show the effect of CAFD 5, 10, and 20% on the brain levels of proinflammatory cytokines (TNF-α and IL-6) induced by LPS in mice. One-way ANOVA indicates that there were significant changes in the concentration of TNF-α (Fig. 9a) and IL-6 (Fig. 9b) levels after LPS treatment relative to BD controls. However, post hoc analysis by Newman–Keuls test showed that CAFD significantly ($p < 0.001$) reduced LPS-induced increase brain level of TNF-α (Fig. 9a) and IL-6 (Fig. 9b).

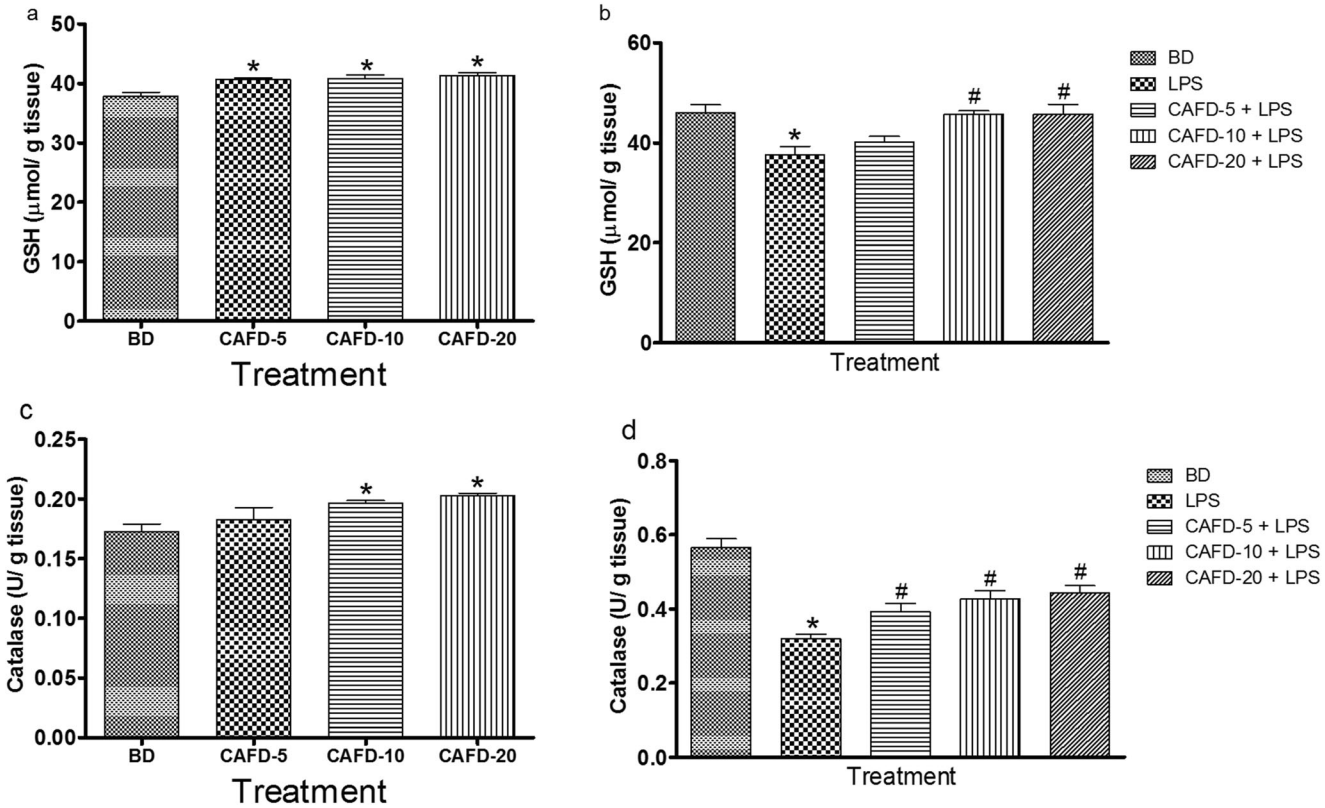

Fig. 7 *Chrysophyllum albidum* fruit-supplemented diet increases glutathione and catalase levels in naïve (**a**, **c**) and LPS-induced alteration in glutathione and catalase levels (**b**, **d**) in mice brains. Data are expressed as mean ± SEM ($n = 6$). *$p < 0.05$ vs BD; #$p < 0.05$ compared with LPS (one-way ANOVA followed by Newman–Keuls post hoc test). BD basal diet, LPS lipopolysaccharide, CAFD *Chrysophyllum albidum* fruit-supplemented diet at 5%, 10%, and 20% supplementation

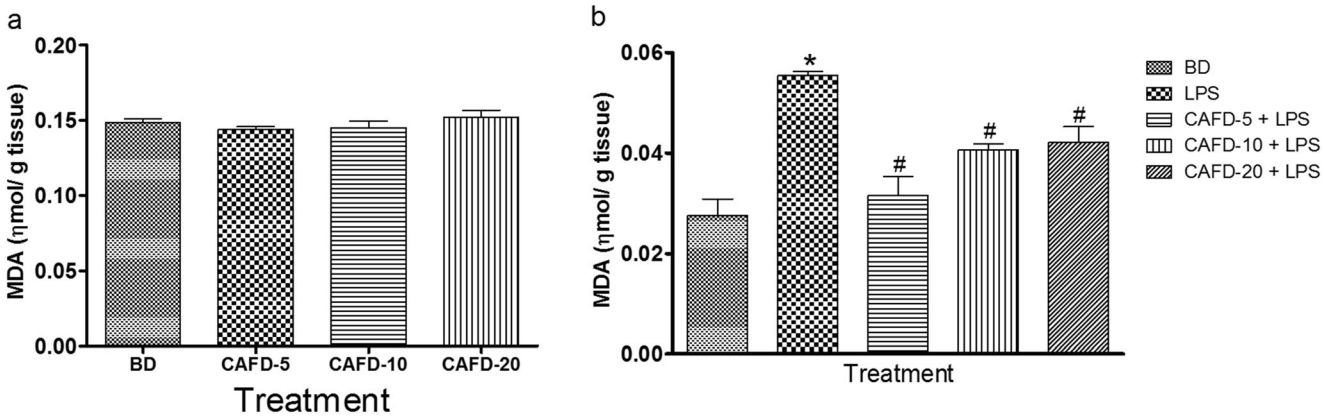

Fig. 8 Effects of *Chrysophyllum albidum* fruit-supplemented diet lipid peroxidation levels in naïve (**a**) and LPS-treated (**b**) mice brains. Data are expressed as mean ± SEM ($n = 6$). *$p < 0.05$ vs BD; #$p < 0.05$ compared with LPS (one-way ANOVA followed by Newman–Keuls post hoc test). BD basal diet, LPS lipopolysaccharide, CAFD *Chrysophyllum albidum* fruit-supplemented diet at 5%, 10%, and 20% supplementation

Discussion

To better understand the role of diet and its effect on cognitive function, the present study employed supplementation with *C. albidum* fruit already shown to possess antioxidant and anti-neuroinflammatory properties [25]. Oxidative stress and neuroinflammation is a characteristic feature of neurodegenerative disorders. In this study, we used the LPS-induced oxidative stress and neuroinflammation model of mice since the molecular mechanisms of LPS-induced neurotoxicity are very much similar to that of neurodegenerative disorders, including AD [39]. Accordingly, the present study showed that CAFD possesses functional neuropharmacological effects as evidenced by prevention of LPS-induced behavioral changes. Also, CAFD increased antioxidant activity and normalized LPS-induced oxidative stress and neuroinflammation.

Behavioral studies are of great importance in the systematic analysis of cognitive changes as well as emotional state associated with neuro-oxidation, neuroinflammation, and neurodegeneration. In the present contribution, YMT, NORT, and SRM were employed to analyze various aspects of cognitive functions. Also, activity meter cage (open field test (OFT)) was used in the assessment of motor function of emotional behavior [32]. Previous studies have shown that OFT measures emotional motor behavior based on aversive condition because of factor of novelty [40, 41]. Following the fact that reduced motor activity due to emotional disturbance is commonly associated with the cognitive dysfunction of some neurodegenerative diseases, our findings that CAFD (10%) supplementation prevents LPS-induced locomotor suppression suggests a beneficial effect in certain cognitive disorders associated with motor impairment. Y-maze is yet another validated test method widely employed for investigating the learning and memory functions associated with various drugs. The Y-maze test is based on the ability of rodents to remember the sequence of arms entry, commonly known as spontaneous alternations [42]. Thus, the list of arms visited is believed to be held in spatial working memory, thereby preventing revisits to the previous arm. Typically, rodents always remember the last arm visited in order to alternate the arm choice, thus serving as a measure of short-term memory [43]. The CAFD 20% showed significant percent correct alternation as evidence of improved memory performance in mice on the Y-maze test. Also, the results of this present study showed that

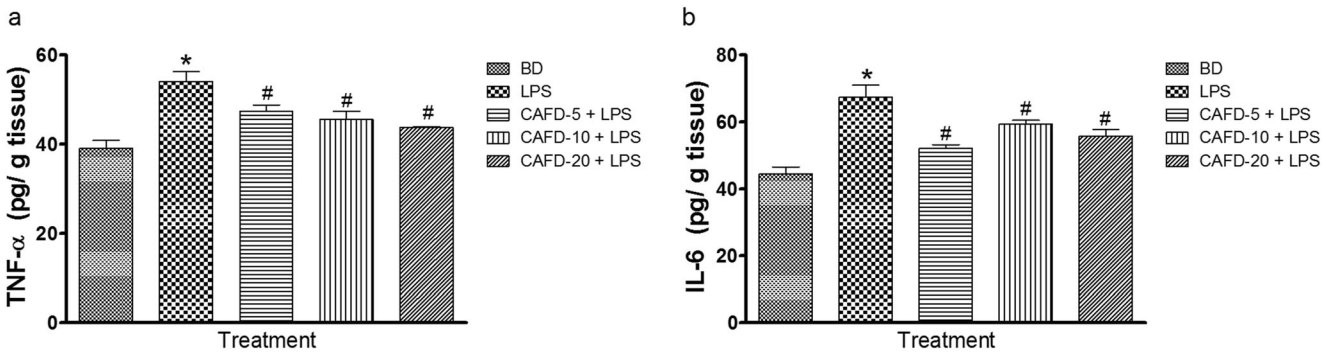

Fig. 9 *Chrysophyllum albidum* fruit-supplemented diet (CAFD) decreases brain TNF-α (**a**) and IL-6 (**b**) levels in mice brains submitted to lipopolysaccharide. Data are expressed as mean ± SEM ($n = 6$). *$p < 0.05$ vs BD; #$p < 0.05$ compared with LPS (one-way ANOVA followed by Newman–Keuls post hoc test). BD basal diet, LPS lipopolysaccharide, CAFD *Chrysophyllum albidum* fruit-supplemented diet at 5%, 10%, and 20% supplementation

i.p. injection of LPS produced a decrease in alternation behavior of mice in Y-maze paradigm, which is consistent with previous studies and thus, indicates memory impairment [44–46]. However, CAFD did not show any significant correction of the LPS-induced alteration in spatial working memory relative to control.

The effect of CAFD on memory performance was further assessed in mice using NORT. The NORT measures non-spatial working memory and takes advantage of mice unprompted nature to explore its natural surroundings [47]. Traditionally, the usefulness of NORT in the evaluation of memory function is based on the natural preference of rodents for novel objects. The preference for a novel object over the familiar one is an indication of the existence of the familiar object in the memory of the animals. Thus, the parameter, which indicates memory performance in NORT, is based on the increase in the amount of time spent on exploration of novel objects in comparison with familiar objects. However, the extent of exploration depends on the amount of residual memory of the familiar object. In this test, CAFD increased the amount of time spent on exploration of the novel object, which suggests enhancement of non-spatial working memory. In addition, the impaired preference for novel object induced by LPS was significantly reversed by CAFD, which further suggests its efficacy in cognitive dysfunctions associated with non-spatial memory deficits.

Furthermore, the effect of CAFD on sociability recognition memory was assessed using social recognition memory test, which is based on interactive learning of complex stimuli in a social environment that enables the organisms to discriminate between familiar and unfamiliar individuals [48]. Accordingly, patients with advanced AD have difficulty in recognizing friends and family members, which makes social recognition memory an important component of social interaction [49]. Preclinically, it has been reported that animals tend to recognize each other on the basis of multimodal sensory characteristics, conjunctively programmed in their recognition memory [50]. Basically, memory function in this test is judged by the amount of time spent by mouse in exploring a counterpart mouse upon re-exposure relative to initial presentation. Increased investigation time of a counterpart mouse in subsequent exposure suggest impaired memory [9, 48]. Compounds that enhance cognitive performance have been reported to reduce social investigation time following re-exposure to a conspecific familiar mouse, which further confirmed the existence of the residual recognition memory of the counterpart mouse [9]. In this investigation, CAFD significantly decreased the period of exploration of conspecific familiar mice following re-exposure, thus indicating enhancement of social recognition memory. This enhancement might be based on modulation and enhancement of long term potentiation via mechanism associated to increased top-down control of glutamatergic system [48].

Pharmacological evaluation of memory functions usually involves both behavioral and biochemical approaches. The biochemical approach involves evaluation of the underlying pathological processes that may influence the behavioral component of memory functions [10]. The biochemical assays carried out in this study revealed that CAFD decreased AChE and increased antioxidant activities in the mouse brain. Inhibition of acetylcholinesterase activity leads to increased brain levels of acetylcholine (ACh), the neurotransmitter agent that plays an essential role in memory and learning processes [10]. Notably, the use of acetylcholinesterase inhibitors, like donepezil, rivastigmine, and galantamine, which act by increasing the availability of ACh in the brain for the symptomatic treatment of AD, has failed to alter the course of the disease and their clinical efficacy has also been compromised by the incidence of toxicity, therefore the search for more effective and safer agents. Thus, in the context of the present study, it is worthy to note that CAFD supplementation was found to be very safe when given to mice, as no toxic effect was observed throughout the feeding period. Moreover, CAFD forms an important component of the human diet. It is known that the intricate and concerted activity of neurotransmitters, their receptors and degrading enzymes form the basis for neuronal communication system; the basis of a healthy cognitive function. ACh, an important chemical messenger responsible for memory, has been shown to have its levels regulated by the hydrolytic enzyme, AChE in both the periphery and brain tissues. Hence, AChE activity in the brain is an acceptable marker of cholinergic activity and progression of AD [51]. When treated with LPS, AChE activity in the mice brain was significantly increased, indicating a decrease in cholinergic activity and concurrent memory deficit as evidenced in this study [10]. However, a significant improvement was observed with the supplementation of CAFD. In the perspective, it might be speculated that the mechanism involved in memory enhancing effect of CAFD might be mediated partly through inhibition of AChE activity. Moreover, both preclinical and clinical studies have shown a close association between inhibition of AChE activity and improvement of cognitive functions [52].

Furthermore, the results of this study showed that *C. albidum* fruit supplementation in diet of mice improve memory performance by reducing oxidative stress and inflammatory process in LPS-treated mice. It is generally accepted that oxidative stress plays a pivotal role in the pathogenesis of memory deterioration. Both preclinical and clinical studies have shown increased level of oxidative stress during latent period of the disease, which often leads to sudden onset of symptoms of cognitive decline [53]. Increased generation of ROS may occur from drug treatment, certain diseases and normal aging of the organism [52]. Brain tissue in particular is more susceptible to the deleterious effects of ROS because of its high rate of oxygen consumption, high levels of

polyunsaturated fatty acids (PUFAs), and reduced antioxidant defense systems [54]. Several studies have shown that ROS causes lipid peroxidation, which triggers degeneration of several neuronal cells especially central cholinergic pathways [55]. Postmortem reveals increased levels of MDA, an index of lipid peroxidation in AD brains, which further confirmed the involvement of oxidative stress in the pathogenesis of cognitive dysfunction [53]. In line with this perspective, LPS-induced memory loss has been linked to increased oxidative stress in the whole brain [10], as well as specific regions [9] associated with learning and memory. Also, LPS has been reported to deplete antioxidant molecules like GSH, and therefore further damage brain cells through increased lipid peroxidation [10]. Our findings confirmed that the memory impairment induced by LPS was accompanied by increased oxidative stress, as shown by elevated brain levels of MDA and decreased antioxidant defense systems. Thus, the ability of CAFD to reverse LPS-induced memory impairment in mice suggests an action involving inhibition of oxidative stress in the brain. This action might have resulted from its ability to overcome the pro-oxidant effects of LPS in the brain, through increases in antioxidant defense systems (GSH and catalase) and a decrease in MDA concentrations [53]. Indeed, it has been suggested that substances with antioxidant property may protect neurons against the injurious effects of ROS and thereby prevent or delay the onset of neurodegenerative diseases such as AD [10].

Recently, LPS-induced memory loss, which closely reflects the pathological changes associated with AD, is increasingly being used as a model for the evaluation of novel compounds with positive effects on cognition [44–46]. LPS-induced memory loss is related to neuroinflammation, which is characterized by activation of microglial cells and expression of several inflammatory mediators in the brain that coordinate degeneration of several cortical neuronal microenvironment especially central cholinergic systems [44–46]. Neurodegeneration and cognitive deficits induced by LPS has also been linked to increased oxidative stress, which further promotes over expression of cytokine storms. It has been reported that neuroinflammation occurs in response to inflammatory challenges and is mediated by release of pro-inflammatory molecules including cytokines and prostanoids, within the brain parenchyma [56]. Interleukin-6, for example, has been established as a powerful pro-inflammatory cytokine with pleiotropic physiological and behavioral functions. Studies have shown that IL-6 activates microglia and increases blood–brain barrier permeability, which encourages leukocyte infiltration and up-regulation of other pro-inflammatory molecules including TNF-α [57]. Previous clinical studies have reported elevated brain levels of IL-6 and TNF-α in patients with AD, which perhaps establishes a causative link between IL-6 brain levels and memory deficits [56]. To this end, the findings from our studies also confirmed the results of previous investigations, which showed that i.p. injection of LPS increased the levels of TNF-α and IL-6 [9, 10]. Indeed, increase in brain concentrations of IL-6 and TNF-α have been implicated in disruption of neuronal functions and memory deficits [44–46]. However, CAFD was found to reduce elevated brain levels of TNF-α and IL-6 induced by LPS in mice, which might be playing a significant role in its memory promoting effect. Thus, these findings revealed the potential benefits of CAFD in neurological disorders associated with memory deteriorations and neuroinflammation as the major underlying factor.

Conclusion

Chrysophyllum albidum fruit supplementation in diet enhances memory function and prevents cognitive deficits induced by lipopolysaccharide in mice. These functional beneficial effects might be due to mechanisms associated with inhibition of oxidative stress-related processes, acetylcholinesterase activity, and pro-inflammatory mediators. Thus, supplementation with *C. albidum* fruit in this study may confer neurocognitive benefit and thus serves as a recommendation for the consumption of African star apple as a preventive diet for cognitive impairments, as well as establish a basis for more comprehensive trials to study the preventive potential and neuronal mechanisms involving memory loss using dietary modifications and supplements.

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Availability of data and material Not applicable.

Code availability Not applicable.

Author contributions AMA and SU designed the experiment; AMA, EOC, and BB performed the experiment and wrote the draft manuscript; all authors approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval The ethical approval for the animal care and use procedure for the experiment was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee with approval number - UI-ACUREC/18/0054.

Consent to participate Not applicable.

Consent to publication Not applicable.

Abbreviations *CAFD, Chrysophyllum albidum* fruit diet; *LPS,* Lipopolysaccharides; *YMT,* Y-maze test; *NORT,* Novel object

recognition test; *OFT,* Open field test; *NCD,* Non-communicable diseases; *TNF,* Tumor necrosis factor; *IL-6,* Interleukin-6

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