Astaxanthin Ameliorates Doxorubicin-Induced Cognitive Impairment (Chemobrain) in Experimental Rat Model: Impact on Oxidative, Inflammatory, and Apoptotic Machineries

Sara Emad El-Agamy 1 · Amal Kamal Abdel-Aziz 1 · Sara Wahdan 1 · Ahmed Esmat 1 · Samar S. Azab 1

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Abstract Chemobrain refers to a common sequelae experienced by 15–80% of cancer patients exposed to chemotherapeutics. The antineoplastic agent doxorubicin (DOX) has been implicated in a strenuous neurotoxicity manifested as decline in cognitive functions, most probably via cytokine-induced oxidative and nitrosative damage to brain tissues. Astaxanthin (AST), a naturally occurring carotenoid, is reputable for its outstanding antioxidant, anti-inflammatory, and antiapoptotic activities. Therefore, the aim of the current study was to investigate the potential neuroprotective and memory-enhancing effects of AST against DOX-induced behavioral and neurobiological abnormalities. Briefly, AST treatment (25 mg/kg) significantly protected against DOX-induced memory impairment. Furthermore, AST restored hippocampal histopathological architecture, halted DOX-induced oxidative and inflammatory insults, mitigated the increase in acetylcholinesterase activity, and consistently downregulated the overactive apoptotic machineries. In conclusion, these findings suggest that AST offers neuroprotection against DOX-induced cognitive impairment which could be explained at least partly by its antioxidant, anti-inflammatory, and antiapoptotic effects.

Keywords Chemobrain · Doxorubicin · Astaxanthin · Oxidative stress · Neuroinflammation · Apoptosis

Introduction

Chemobrain or the cognitive impairment resulting from chemotherapeutic treatment was recently recognized by the National Cancer Institute as one of the most troublesome morbidities for cancer survivors that devastatingly diminishes their quality of life and averts them from reclaiming their pre-cancer life [1–3]. Chemobrain implicates symptoms such as memory impairment, slow processing speed, inability to concentrate, and language difficulty, which mainly declare the loss of integrity of the hippocampus together with the frontal systems [4].

Breast cancer survivors are the most frequently affected class of patients [5, 6]. Importantly, doxorubicin (DOX) has been shown to be often encompassed within the antineoplastic regimens of breast cancer survivors suffering from chemobrain [7]. DOX, a member of the anthracycline topoisomerase II inhibitors, is one of the most effective and fundamental antineoplastic agents approved by the FDA to treat several types of cancer [8, 9]. Despite its outstanding clinical effectiveness, DOX provokes multi-organ toxicity in the form of fatal cardiotoxicity, hepatotoxicity, nephrotoxicity, and a troublesome neurotoxicity which hampers its avail [10–14]. Since it is known to be incapable of crossing the blood–brain barrier (BBB), the mechanism underlying DOX-induced chemofog is presumed to be a cytokine-induced oxidative and nitrosative damage to the brain tissues [15–17]. Accordingly, given the increasing number of cancer survivors suffering from “DOX-induced chemobrain,” there is an ultimate need to manage and even prevent such life-debilitating cognitive impairment without compromising DOX’s anticancer efficacy.

Over the last few years, there has been a growing interest in investigating the therapeutic utility of astaxanthin (AST), a non-provitamin A carotenoid that is widely found in several

1 Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt

* Samar S. Azab

samar_saad_azab@pharma.asu.edu.eg
marine organisms [18]. By virtue of its potent antioxidant capacity and its favorable safety profile, the US FDA has lately approved AST to be used as a dietary supplement [19]. Moreover, AST exhibits anti-inflammatory and antiapoptotic activities [20–22]. In addition to its cardio- and hepatoprotective effects, AST has been proven to be an effective neuroprotective candidate in various experimental models of neurological diseases [21, 23–26]. Of major interest, it has lately been reported that AST both augmented DOX’s anticancer activity and guarded against its nephrotoxicity [27, 28]. Accordingly, the aim of the present study was to determine whether AST could confer a neuroprotective effect against DOX-induced chemobrain and elucidate its underlying molecular mechanisms in terms of oxidative stress and inflammatory, apoptotic, and acetylcholinesterase modulatory arbiters.

### Materials and Methods

#### Animals

Male albino rats (150–200 g) were purchased from an animal breeding facility (National Research Center, Giza, Egypt). The animals were housed in an air-conditioned chamber (24±2 °C) with alternating 12-h day/night cycles and allowed access to standardized food pellets and water ad libitum and left for 1 week to acclimatize prior to starting the experiment. Standardized food pellets contained the required amounts of protein, fiber, and fat together with a vitamin mixture to provide the required level of metabolic energy. All efforts were made to minimize animal suffering and reduce the number of animals used.

#### Drugs and Chemicals

Doxorubicin was purchased as doxorubicin hydrochloride from Shaanxi Xinheng Biotech Co., Ltd. (Shaanxi, China). Astaxanthin (98%) was purchased from the CN Lab Nutrition, Asian Group (Shaanxi, China). Acetylthiocholine iodide (Cat. No. A5751) and 5,5′-dithiobis-(2-nitrobenzoic acid) (Cat. No. D8130) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The following antibodies were purchased from Thermo Fisher Scientific Co., UK: anticytochrome c (Cat. No. PA1-29157) and antiglial fibrillary acidic protein (Cat. No. PA3-16727). All other chemicals and solvents used were of the highest purity grade commercially available.

#### Experimental Design

Rats were randomly assigned into four groups and treated for 4 weeks as follows:

- The first group served as the control group and received the following vehicles: saline and olive oil.
- The second group served as the DOX-treated group and received DOX dissolved in saline (2 mg/kg/week, i.p.). DOX dose was chosen according to previous studies [29, 30] and also was confirmed by the results of neurobehavioral experiments and histopathological examination that we obtained from a preliminary pilot study performed in our lab.
- The third group served as the treatment group and received AST suspended in olive oil (25 mg/kg/day, P.O., 5 days per week), concomitantly administered with DOX (2 mg/kg/week, i.p.). AST dose was chosen based on previous studies [31, 32] and also was confirmed by the results of neurobehavioral experiments and histopathological examination that we obtained from a preliminary AST dose-response study performed in our lab.
- The fourth group served as the drug alone-treated group and received AST alone (25 mg/kg/day, P.O., 5 days per week). Behavioral tests and decapitation were conducted as shown in the timeline (Fig. 1). Rats were euthanized by cervical dislocation, whole brains were dissected out and either stored at −80 °C for neurochemical analyses or fixed in 10% formalin for the preparation of paraffin blocks. Sections form the paraffin blocks were then used for histological examination, toluidine blue staining, and immunohistochemical detection of glial fibrillary acidic protein (GFAP) and cytochrome c.

#### Behavioral Tests

**Passive Avoidance**

Based on the principle of contextual fear conditioning for assessing memory changes, a step-through passive avoidance apparatus (Ugo Basile, Italy) for rats was utilized to perform the test as previously described [33]. Briefly, the Plexiglas device is divided into two compartments: the first compartment is white and lit up by a 10-W bulb whereas the other one is a black, dark chamber. The grid floor of the latter can be programmed to deliver an electric shock of the required intensity whenever stepped on. The two compartments are partitioned by an automatically sliding door.

Each rat was subjected to two sessions: training and test sessions. During the training session which was performed 1 week after the last dose of DOX [30], rats were gently placed individually in the illuminated chamber. When a rat
stepped through the dark compartment, placing its four paws on the grid floor, the sliding door closed and an electric shock of 1 mA was delivered for 2 s. Rats failing to step into the dark compartment within 90 s were excluded from the experiment.

Test session was carried out 24 h after the training session, in which rats were again, one by one, gently placed in the white compartment and their latency to step through the dark compartment was automatically recorded and considered as a step-through response to evaluate their memory acquisition after being exposed to an aversive stimulus. The cutoff time was set to 5 min; the dark compartment grid was not electrified.

**Locomotion**

An animal activity meter was employed to monitor the locomotor activity of rats (Opto-Varimex-Mini Model B; Columbus Instruments, OH, USA). The principle of measurement depends on the emittance of evenly spaced infrared light beams, where beam interruptions caused by movements of the animal are sensed and counted; hence, the activity of the animal was calculated as the number of movements per 5 min.

**Assessment of Neurodegeneration**

**Histopathological Examination**

Brain samples from different groups were fixed in 10% formal saline (pH = 7.2) for 24 h, then dehydrated with an alcohol gradient. Specimens were then embedded in paraffin at 56 °C in hot air oven for 1 day, then paraffin tissue blocks were sliced at 4 μm thickness using a sledge microtome and the obtained tissue sections were transferred onto glass slides and stained by hematoxylin and eosin stain for histopathological analysis using a light microscope [34].

**Toluidine Blue Staining**

Paraffinized tissue sections of 5 μm thickness were placed on microscope slides, washed with PBS–Triton X-100 (0.4%), and stained with 0.1% toluidine blue for around 2 min. Afterwards, sections were dehydrated and mounted with Canada balsam. Slides were visualized under a light microscope. Hippocampal neurons with rounded nuclei and visible nucleoli were considered viable while those deeply stained shrunken neurons were considered degenerated and were counted by capturing six non-overlapping fields per rat for three rats per treatment group. The number of degenerated neurons/high-power field was expressed as a percentage out of the total neuronal population [34].

**Assessment of Acetylcholinesterase Enzyme Activity**

The assay was performed as previously described by Ellman et al. [35] and Menze et al. [36] with minimal modifications. The enzyme activity was quantified by measuring the concentration of the formed thionitrobenzoic acid and expressed as nanomoles/minute/gram of tissue used.

**Assessment of Oxidative Stress Biomarkers**

**Reduced Glutathione Level**

Glutathione (GSH) assay kit (Cat. No. GR25-11) was purchased from Biodiagnostics Co., (Cairo, Egypt). The assay was carried out as previously described [37], and the results were expressed as micromolars of GSH/gram of tissue used.

**Lipid Peroxidation Product (Malondialdehyde)**

Hippocampal levels of malondialdehyde (MDA), one of the main thiobarbituric acid-reactive substances (TBARS) produced as a result of lipid peroxidation, were assessed using a MDA assay kit (Biodiagnostics Co., Cairo, Egypt; Cat. No. 232x568 to 544x736}
MD25-29), and the results were expressed as nanomoles of MDA/gram of tissue used.

**Catalase Activity**

Catalase activity was assessed using the spectrophotometric method of Aebi [38]. Hippocampal catalase enzyme activity was determined using a catalase assay kit (Biodiagnostics Co., Cairo, Egypt; Cat. No. CA25-17). Briefly, a known amount of hydrogen peroxide was left to react with catalase-containing samples in a buffered medium. Following a fixed incubation period of 1 min, a specific catalase inhibitor was added. The remaining hydrogen peroxide was then quantified by the chromogenic coupling reaction of 4-aminoantipyrine (AAP) and 3,5-dichloro-2-hydroxy-benzenesulfonic acid (DHBS), catalyzed by peroxidase enzyme. The absorbance of the resulting chromophore was measured at 520 nm, and the results were expressed as units/gram of tissue used.

**Assessment of Inflammatory Markers**

**Tumor Necrosis Factor Alpha**

A sandwich enzyme immunoassay technique was used to assess the concentration of tumor necrosis factor alpha (TNF-α) in hippocampal homogenate using a TNF-α ELISA assay kit (Assaypro, St. Charles, MO, USA; Cat. No. ERT2010-1).

**Prostaglandin E2**

Hippocampal prostaglandin E2 (PGE2) levels were determined using a forward sequential competitive technique (PGE2 ELISA assay kit; R&D Systems, Inc., Minneapolis, USA; Cat. No. KGE004).

**Cyclooxygenase-2**

Quantitative determination of hippocampal cyclooxygenase-2 (COX-2) concentration was performed using a rat prostaglandin G/H synthase 2 ELISA kit (EIAab Science Co., Wuhan, China; Cat. No. EO699r).

**Glial Cell Activation**

Hippocampal GFAP levels were evaluated using anti-GFAP antibody through immunohistochemical analysis. Paraffinized brain slides were dewaxed using xylene and then rehydrated using an alcohol gradient. Endogenous peroxidase activity was blocked using a H2O2 solution, and the slides were rinsed with phosphate-buffered saline (PBS). Non-specific binding sites were blocked with a 10% bovine serum blocking buffer, and after 10 min, the remaining blocking buffer was drained. The antibody was added to the sections and incubated overnight at 4 °C, followed by another incubation with a biotinylated secondary antibody, rinsing with PBS twice for 5 min each and a 5-min incubation with DAB substrate solution (0.05% dianinobenzidine–0.01% H2O2 in PBS) to visualize and reveal the color of antibody staining. Images of hippocampal areas were acquired using a light microscope (CX21; Olympus, Japan), and brown areas were quantified as optical density (OD) across six different high-power fields per each rat section for three rats per group (n = 3 rats/group), using ImageJ analysis software (1.46a; NIH, USA).

**Assessment of Apoptotic Markers**

Hippocampal-active cytochrome c levels were evaluated using anticytochrome c antibody using the previously described immunohistochemical analysis paradigm. Caspase-3 activity was assessed using a kit purchased from Sigma-Aldrich (St. Louis, MO, USA; Cat. No. CASP-3-C). The assay depends on the hydrolysis of the provided substrate, acetyl-Asp-Glu-Val-Asp-p-nitroanilide (Ac-DEVD-pNA), by caspase-3 resulting in the formation of p-nitroaniline (pNA) moiety which has high absorbance at 405 nm. Results were expressed as micromoles of pNA/minute/gram of tissue used.

**Statistical Analysis**

Passive avoidance non-parametric data was presented as mean ± SD and analyzed by the Kruskal–Wallis test followed by Dunn’s post hoc test. Parametric data were expressed as mean ± SD and analyzed by utilizing one-way ANOVA followed by the Tukey–Kramer post hoc test. All statistical analyses were implemented using GraphPad software (version 5), and a probability of 0.05 or less was regarded as statistically significant.

**Results**

**AST Reverses DOX-Induced Cognitive Impairment**

In order to assess the potential antiamnestic effect of AST on DOX-induced chemobrain, we performed a step-through passive avoidance (PA) task in different treated groups. Inhibitory avoidance comprises learning to inhibit a response in order to avoid an aversive stimulus. On the training session, there was no statistically significant difference in the step-through latency among different treated groups as shown by the Kruskal–Wallis test. However, during the test session, DOX administration significantly shortened the step-through latency by 93% compared to the vehicle-treated group which denoted evident memory impairment induced by DOX. Interestingly, co-administration of AST significantly reversed DOX-induced amnesia by restoring the normal step-through latency.
Of note, AST alone did not cause any changes in the step-through latency compared to the control group (Fig. 2a).

**AST and/or DOX Does Not Adversely Affect the Locomotor Activity of the Treated Rats**

Although there is no neurochemical correlation between locomotor activity (LA) and PA, LA may have a conclusive effect on the latency of stepping into the shock zone. Hence, in order to exclude the potential impact of any motor disorders on shortening the step-through latency and affirm the latter is solely due to an amnestic effect, we assessed the effect of AST and/or DOX treatment on locomotor activity. One-way ANOVA revealed that there was no statistically significant difference between different treated groups (Fig. 2b).

**AST Dramatically Ameliorates DOX-Induced Hippocampal Neurodegenerative Changes**

Histopathological examination revealed that systemic DOX administration resulted in nuclear pyknosis and degeneration in most of the neuronal cells of the subiculum, fascia dentata, and hilus of the hippocampus in association with focal hemorrhage in the area separating the hippocampus from the striatum. In contrast, co-administration of AST and DOX completely restored the hippocampal histological features. Vehicle- and AST only-treated groups had normal histological structures of the hippocampus, cerebral cortex, striatum, cerebellum, and medulla oblongata (Fig. 3a). The extent of hippocampal neurodegeneration was further assessed using toluidine blue stain. As shown in Fig. 3b, the hippocampal neuronal population of DOX-treated group showed a complete loss of the histological architecture as evidenced by the presence of hyperchromatic neurons with darkened cytoplasm and pyknotic nuclei altogether with a loss of the rounded framework of normal neurons. Strikingly, DOX significantly increased the number of degenerated hippocampal neurons by 311% compared to the vehicle-treated group (Fig. 3c). Conversely, AST co-treatment restored the normal neuronal features manifested by the rounded neuronal build, euchromatic cytoplasm, and defined nuclei. AST alone-treated group showed no deviation from the normal histological architecture of the vehicle-treated group.

**Effect of AST on Hippocampal AChE Activity in DOX-Treated Rats**

As shown in Fig. 4, DOX administration increased acetylcholinesterase (AChE) activity by 60% compared to the vehicle-treated group. On the other hand, AST co-treatment precluded any alteration in the enzyme activity.

**AST Reverts DOX-Induced Hippocampal Oxidative Stress**

In order to assess the effect of AST and/or DOX treatment on hippocampal oxidative stress status, we assessed the effect on the levels of reduced GSH and MDA as well as catalase activity among treated groups. Administration of DOX at a dose of 2 mg/kg/week for four consecutive weeks significantly triggered a pro-oxidant effect as evidenced by dramatic depletion of reduced GSH levels, elevation of MDA levels, and reduction of catalase activity by 65, 53, and 72%, respectively, compared to the vehicle-treated group. In contrast, co-administration of 25 mg/kg AST with DOX prominently restored normal MDA and GSH levels as well as catalase activity. Moreover, AST alone-treated group showed significant reduction in the level of MDA by 29% compared to the control group (Table 1).
The Anti-inflammatory Capacity of AST Abrupt DOX-Induced Pro-inflammatory Response in Hippocampal Tissues

Assessment of the potential modulatory effect of AST on pro-inflammatory cytokines and enzymes revealed that DOX triggered robust inflammatory response in the hippocampal tissues as shown by the statistically significant upsurge of TNF-α, PGE2, and COX-2 levels by 110, 211, and 85%, respectively, compared to the control group. In contrast, AST co-treatment significantly attenuated heightened inflammatory response as manifested by the restoration of the normal levels of the assessed parameters. AST alone-treated group showed no statistically significant difference from the vehicle-treated group (Fig. 5a–c). Furthermore, immunohistochemical detection of GFAP revealed minimal immunoreactive staining within both vehicle- and AST alone-treated groups. DOX-treated rats showed a significant increase in immunoreactivity by 66% that came to halt upon AST co-administration (Fig. 6a, b).

Effects of AST on Hippocampal Apoptotic Markers in DOX-Treated Rats

Immunohistochemical analysis revealed minimal expression of hippocampal cytochrome c in the control sections. Likewise, rats treated with AST alone for 4 weeks exhibited an immunoreactivity analogous to the control group. Contrariwise, DOX administration induced a remarkable increase in the expression of the apoptotic marker, cytochrome c, by 57% as evidenced by the increase of the intense brown patches of positive cells dispersed along the whole hippocampal area. Notably, co-administration of AST significantly suppressed its expression (Fig. 7a). Quantitation of immunohistochemical staining was assessed as optical density of the stained regions (Fig. 7b). Antiapoptotic activity of AST was further confirmed using a CASP3C kit where AST treatment dramatically reverted DOX-induced activation of the execution caspase-3 by 642% (Fig. 7c).

Discussion

Doxorubicin (DOX) is considered a milestone in cancer therapy. In addition to its cardiotoxic, nephrotoxic, and hepatotoxic insults, DOX-induced cognitive impairment or “chemobrain” has vastly been reported by cancer survivors, which adversely affected their quality of life. Consistently, in the present study, systemic DOX administration in male albino rats recapitulated clinically reported DOX-induced chemofog as evidenced by significant shortening in the step-through latency in passive
avoidance paradigm without affecting the locomotor activity. Our findings are also in agreement with previous studies which reported that DOX administration impaired contextual and cued fear conditioning performance and spatial memory in rats [29, 30]. Since memory acquisition, consolidation, and retrieval are mainly the functions of the hippocampus, in line with the neurobehavioral data, histopathological examination and toluidine blue staining revealed severe nuclear pyknosis in the hippocampal tissues of DOX-treated rats. Intriguingly, AST co-treatment halted DOX-induced cognitive deficits at both neurobehavioral and histopathological levels. Similarly, AST guarded against aluminum chloride and diabetes mellitus-induced memory impairment [39, 40]. Moreover, preliminary data from clinical trials suggested that AST-treated subjects with cognitive deficits displayed better scores in performances on cognitive tests when compared to their own baseline data [41].

Given the fact that DOX is incapable of crossing the BBB, yet impairs memory processing emphasizes that crossing the BBB per se is dispensable and thereby hints for an indirect ovement through which a peripherally confined chemotherapeutic agent can elicit central neurotoxicity [42]. In this context, it has been suggested that oxidative stress may play a master role in DOX-induced cognitive impairment [43]. DOX is a quinone-containing molecule that undergoes a process of redox cycling, in which a single electron is introduced to the quinone moiety forming a semi-quinone intermediate which is recycled back to the parent quinone at the expense of producing massive amounts of reactive oxygen species (ROS), thus sabotaging the structural and functional integrity of several pivotal biomolecules. In the present study, the amnestic effect induced by systemic DOX administration for 4 weeks was associated with heightened oxidative stress in hippocampal tissues as depicted by significant elevation of the level of lipid peroxidation product (MDA) and reduction of both reduced GSH level and catalase activity.

Likewise, it has been shown that the neurotoxic effects of DOX are secondary to increased lipid peroxidation and protein oxidation [44], reduced GSH levels, and dysregulated activity of various delicately balanced antioxidant enzymes [45] including that of the most important mitochondrial antioxidant enzyme, manganese superoxide dismutase (MnSOD). Furthermore, ROS also react with nitric oxide-producing peroxynitrite (ONOO–), which is regarded as one of the most harmful reactive nitrogen species (RNS), as it nitrates the tyrosine residues in proteins, hindering post-translational modifications and thereby hampering vital signaling pathways [17, 46]. Interestingly, relying on AST’s unique molecular layout that allows it to quench free radicals from the hydrophobic cell membrane along with its hydrophilic interior and its outstanding ability to induce the expression of several antioxidant enzymes [21, 47, 48], the present findings also implied that the neuroprotective effects of AST in halting DOX-induced chemobrain could be at least partially attributed to the antioxidant capacity of AST.

Despite the postulated cardinal role of ROS in mediating doxorubicin neurotoxicity, the aforementioned mechanism is not veracious for introducing ROS into the brain because DOX is incapable of crossing the BBB [49]. This hint therefore implies the potential implication of another indirect mechanism that does not necessarily encompass redox cycling within the CNS. In this context, it has been shown that ROS yielded from a redox cycling process activates the redox-responsive transcriptional factor, nuclear factor kappa B (NF-κB), which, in turn, activates the transcription of multiple

Fig. 5 Effect of AST treatment on DOX-induced inflammatory response in hippocampal tissues. a TNF-α, b PGE2, c COX-2. Data are presented as mean ± SD (n = 10). Statistical analysis was carried out using one-way ANOVA followed by Tukey’s post hoc test. Asterisk and number sign indicate statistical significance from the control and DOX-treated groups, respectively (P < 0.01)
target genes encompassing inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [50]. So, together with the striking findings reported by Hayslip et al., who observed a prominent increase in plasma TNF-α levels in treated cancer patients 6 h following DOX administration among multi-agent chemotherapy regimens [3], attention was directed towards another key player, namely inflammation. Inflammation appears to be a ubiquitous hallmark, with cytokines being the main perpetrator of DOX-induced cognitive impairment [7].

Of the pro-inflammatory mediators released, TNF-α is blamed for disrupting the integrity of the BBB resulting in an uninterrupted feedback loop of inflammation between the periphery and the CNS [51]. It is also capable of inhibiting long-term potentiation in the hippocampal CA1 and the dentate gyrus regions [52]. Indeed, in our current study, DOX administration dramatically increased TNF-α, PGE2, and COX-2 levels in the hippocampal tissues of treated rats emulating heightened neuroinflammatory response. Such effects could be attributed to DOX’s pro-oxidant capacity since it has been shown that ROS leads to apolipoprotein A1 (Apo-AI) oxidative modification [53]. Apo-AI is a multi-functional apolipoprotein that plays multiple roles in normal body functioning including regulation of inflammatory response through depressing the production of inflammatory cytokines, mainly TNF-α, by increasing the production of a messenger RNA (mRNA)-destabilizing protein called tristetraprolin which reacts with TNF-α mRNA, prompting the latter degradation and consequently inhibiting TNF-α translation [54]. Accordingly, oxidative modification of Apo-AI lipoprotein abolishes its anti-inflammatory activity ensuing an immense liberation of cytokines that are capable of crossing the BBB [55], amplifying localized transcription of iNOS and COX-2 enzymes resulting in increased production of NO and hence ROS/RNS and prostanooids, respectively [56]. In line with our data, strong TNF-α immunoreactivity was reported in the hippocampus of DOX-treated mice [15].
Astrocytes, the most abundant neuroglia, is habitually involved in regulating synaptic transmission, neurovascular coupling, cerebral microcirculation, as well as both constituting and preserving the integrity of the BBB [57–59]. A considerable body of evidence suggests that astrocytes play an indispensable role in neuroinflammation by responding to insults from pro-inflammatory cytokines evoking a state of astrogliosis in which astrocyte activity is overturned from beneficial into detrimental being chaperoned with superfluous downstream release of inflammatory signals causing a vicious endless cycle of deleterious neuroinflammation that promotes oxidative and nitrosative stress and reduces neuroplasticity [60]. Similarly, increased level of GFAP, the hallmark of astrogliosis [61], was observed in the hippocampal tissues of DOX-treated rats, further confirming progressive hippocampal neuroinflammatory response. In contrast, AST treatment evidently halted such inflammatory cascade which may be attributed to its ability to inhibit astrocyte activation in hippocampal tissues [62]. Moreover, the anti-inflammatory action of AST could be due to its ability to halt ROS-induced activation of NF-κB signaling pathway through inhibiting IκB-α subunit degradation and p65 nuclear translocation along with suppressing genetic expression of inflammatory mediator enzymes [20, 63–67].

Taking into consideration the fact that cholinergic neurons play an imperative role in hippocampal-dependent learning and memory and critical loss of acetylcholine in the CNS has linked to the pathogenesis of various dementia-associated disorders including Alzheimer’s and Parkinson’s diseases [68–70]. Thus, we assessed the potential effect on AChE activity as a marker of cholinergic dysfunction. Surprisingly, boosted AChE was detected in the hippocampal tissues of DOX-treated rats which could be due to cytokine-induced oxidative and nitrosative stress imposed by DOX. Since AChE inhibition is regarded as a mainstay strategy for treating cognitive deficit-associated disorders, we questioned whether AST could modulate AChE activity and, indeed, AST treatment restored AChE activity in DOX-treated rats. This could be, at least in part, due to AST’s potent antioxidant activity [71]. To the best of our knowledge, this is the first study to report both the positive and negative neuromodulatory effects of DOX and AST, respectively, on hippocampal AChE activity. Yet, it needs to be further elucidated whether such regulatory effects are directly or indirectly mediated.

Since neurons are post-mitotic cells, activation of apoptotic signaling pathways is an irremediable event that could ultimately be a substantial contributor to chemotherapy-induced cognitive impairment. This suggests that reinforced apoptosis presumably preceded atrophy of those areas. In this regards, it has been shown that DOX increases the susceptibility of the mitochondria to calcium-mediated permeability transition pore opening leading to mitochondrial swelling, membrane degradation, and release of apoptotic proteins, eventually predisposing to neuronal degeneration [15, 16, 72]. Conversely, AST distinguishesably preserves mitochondrial integrity through upregulating the expression and activity of antiapoptotic Bcl-2 and decreasing pro-apoptotic Bax, cytochrome c, caspase-3, and caspase-9 expression [24, 73, 74]. This is in accordance with our findings where AST was able to preclude DOX-mediated mitochondrial dysfunction as evidenced by restoring normal cytochrome c level and caspase-3 activity in hippocampal tissue. The aforementioned mitochondrial abnormalities could be explained by Tangpong et al., who showed that TNF-α-neutralizing antibodies completely abrogated the mitochondrial injury in the brain tissues [15], which aggrandize the role of this specific pro-inflammatory cytokine in DOX-induced chemobrain, an annotation that was previously proclaimed by Usta et al., who demonstrated that pentoxifylline, a TNF-α inhibitor, voided DOX nephrotoxicity [75].

Conclusion

The present study provides evidence that the naturally occurring carotenoid AST could present a promising antiamnestic and neuroprotective agent which could guard against DOX-induced cognitive deficits. Furthermore, we highlighted that the underlying molecular mechanisms beyond the cognitive boosting effects of AST as evidenced by neurobehavioral tests, toluidine blue staining and histopathological examination, could be at least partly attributed to a myriad of actions including its antioxidant, anti-inflammatory, and antiapoptotic activities (Fig. 8). Accordingly, the current study serves as an impetus supporting clinical investigation of the neuroprotective impact of AST supplementation in DOX-treated cancer patients.

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Compliance with Ethical Standards The experimental protocol including procedures of laboratory animal care in research was approved by the Research Ethics Committee, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt, under the memorandum no. 87.
Conflict of Interest

The authors declare that they have no conflict of interest.

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