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ORIGINAL ARTICLE

Soursop fruit extract mitigates scopolamine-induced amnesia and oxidative stress via activating cholinergic and Nrf2/HO-1 pathways

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Abstract

Current therapeutic interventions for memory loss are inadequate and are associated with numerous adverse effects. There is an urgent need for new alternative agents for the treatment of memory loss and related disorders. Here, we investigated the potential neuroprotective role of soursop fruit extract (SSFE) in scopolamine (SCO)-induced amnesia and oxidative damage in the hippocampus of rats. Thirty-five rats were randomly allocated into 5 groups: control, SCO, SSFE, SCO, SSFE+SCO and N-acetylcysteine (NAC) + SCO. SCO-treatment increased acetylcholine esterase activity and decreased hippocampal levels of acetylcholine, serotonin, dopamine, norepinephrine, and histamine. The level of ATP increased. SCO-treated rats showed a disturbance in oxidative status, which was evident through the increase in malondialdehyde, and nitrites/nitrates and a decrease in cellular antioxidant molecules including glutathione, superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase. A disturbance was also observed via downregulation of the nuclear factor erythroid 2–related factor 2 and heme oxygenase-1 defense pathways. SCO-treatment enhances a neuroinflammatory state, as indicated by the release of tumor necrosis factor- α and interleukin-1β and increased inducible nitric oxide synthase and mRNA expression. SCO-treatment decreased the expression of the anti-apoptotic protein, B cell lymphoma 2 and increased the expression of the pro-apoptotic protein, Bcl-2 associated X protein, caspase-3 and cytochrome *c* in hippocampal neurons. SSFE pretreatment markedly ameliorated hippocampal changes. Our findings revealed that SSFE exerts its potential anti-amnestic effect mainly through the activation of the cholinergic system and Nrf2/HO-1 pathway.

Keywords Soursop · Scopolamine · Cholinergic system · Oxidative stress · Neuroinflammation · Nrf2/HO-1 pathway

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Introduction

Alzheimer's disease (AD) is the most common chronic neurodegenerative disorder amongst older people in industrial countries. Development of AD is associated with severe cognitive impairments, behavioral and psychiatric deterioration including: agitation, depression, hallucinations, and a decline in basic daily activities including eating without help, dressing and toileting (Chhetri et al. 2018; Moneim 2015). AD is the most common form of dementia, representing approximately 60% of memory impairment cases worldwide (Sosa et al. 2012). The precise mechanism of AD is unclear. However, cholinergic dysfunction, senile plaques and neurofibrillary tangles deposition in the brain tissue have been identified as hallmarks of AD progression (Kumar et al. 2015). Other mechanisms may be involved in the development of AD including oxidative reactions, neuroinflammation and apoptosis (Moneim 2015).

Scopolamine (SCO) is used to induce amnesia in experimental animals as it antagonizes muscarinic acetylcholine (ACh) receptors thus, increasing acetylcholinesterase (AChE) activity in cortical and hippocampal neurons leading to memory and learning deficits. SCO has been used in experimental models to evaluate the efficiency of potential anti-amnesic drugs (Lee et al. 2017).

Current anti-amnesic medications are mainly AChE inhibitors that alleviate cognitive dysfunction through the regulation of cholinergic transmission. AChE inhibitors are inadequate and are associated with several adverse effects including: vomiting, nausea, diarrhea, weight loss and hepatotoxicity (Mendiola-Precoma et al. 2016). As a result, there is a growing interest for the development of alternative multi-target and directed natural therapeutic agents for use as anti-amnesic medications (Akram and Nawaz 2017).

In traditional medicine, numerous plants are utilized for the prevention and treatment of different neurological disorders including cognitive impairment and other associated memory and learning diseases (Akram and Nawaz 2017). Soursop or graviola (*Annona muricata* L.) is abundant in tropical regions and belongs to the Annonaceae family. Active ingredients include acetogenins, alkaloids, phenols, vitamins, carotenoids, amides, and cyclopeptides. Different parts of the plant have demonstrated several biological and pharmacological activities including antioxidant, anti-inflammatory, anxiolytic and antidepressant (Moghadamtousi et al. 2015). The role of *A. muricata* extracts in neuroactivity has not been investigated. This study investigated the potential anti-amnesic effect of soursop fruit extract (SSFE) in SCO-induced cognitive deficits through the estimation of AChE activity, ACh and monoaminergic levels, oxidative profile, pro-inflammatory cytokines and apoptotic proteins in the hippocampal tissue of rats.

Materials and methods

Chemicals

Scopolamine hydrobromide was purchased from Sigma (St. Louis, MO, USA). Tris-HCl was obtained from Fluka Chemie (Buchs, Switzerland). Thiobarbituric acid was obtained from Merck (Darmstadt, Germany), nitroblue tetrazolium and phenazine methosulfate were purchased from Alfa Aesar (Tewksbury, MA, USA). Tumor necrosis factor (TNF)-α and interleukin (IL-1β) ELISA kits were obtained from R&D System. TRIzol isolation kit was obtained from Invitrogen (Carlsbad, CA, USA). RevertAid H minus Reverse Transcriptase was purchased from Thermo Fisher Scientific Inc. PCR primers were synthesized by Jena Bioscience GmbH (Jena, Germany). The chemicals and reagents used were all analytical grade.

Plant materials and extraction procedure

The plant was purchased from local area in South-Cairo, Egypt in the month of November 2017. The plant was identified and authenticated by a taxonomy specialist (Botany Department, Faculty of Science, Helwan University, Egypt). The pulps of soursop fruit (*Annona muricata* L.) were powdered using an electrical blinder. The powder crude was macerated three times for 24 h with methanol (70%) and the ratio between the plant powder and methanol was 1:10 (*w*/*v*). The solvent was concentrated under a vacuum evaporator and further lyophilized. The soursop fruit methanolic extract was stored at −80 °C until the beginning of the current study.

Determination of total phenolic content

Folin-Ciocalteu reagent was used for the determination of soursop total phenolic content (TPC) according to the procedures described by Al-Olayan et al. (2014). using gallic acid as a standard. Briefly, 1.0 mL of 10% Folin-Ciocalteu reagent was added to 0.4 mL of the soursop extract with a concentration of 100 μg/mL. 0.8 mL of 7.5% Na_2CO_3 solution was mixed after incubation for 10 min at room temperature. After 2 h, TPC was measured at 765 nm, and then expressed as mg gallic acid equivalents (GAE)/g SSFE.

Determination of total flavonoids content

Total flavonoid content in soursop extract was estimated using the protocol described Ordoñez et al. (2006) using quercetin as a reference. Briefly, 0.1 ml of soursop extract was mixed with 0.3 ml distilled water and after 5 min, 0.03 ml of 5% NaNO2 was added at room temperature. 0.2 ml of 1 mM NaOH was then added to the mixture and finally diluted with 1 ml distilled water. The absorbance was determined at 510 nm and the obtained results were expressed as mg quercetin (QE)/g SSFE.

Animals

Twelve-week-old male Wistar albino rats weighing 150–180 g were obtained from the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Rats were housed in polypropylene cages under continuous 12-h light-dark cycles at temperatures between 20 and 22 °C with 65% humidity. Rats were acclimatized for 10 days prior to the experiment and were fed standard rat pellet chow and had ad libitum access to water. All experiments were conducted according to the ethical standards approved by the Institutional Animal Ethics Committee guidelines for animal care and use, Helwan University, Egypt.

Experimental design

Before the beginning of the current experiment, an acute toxicity study was carried out using a maximum dose of 2000 mg/kg SSFE given orally, which showed no signs of toxicity in rats and the oral dose of SSFE was selected according to a preliminary experiment using three doses of 100, 200 and 300 mg/kg showed that the oral administration of SSFE at a dose of 300 mg/kg was reversed significantly SCO-induced neuronal impairments (data not shown).

Thirty-five rats were divided equally into 5 groups. Group I was administered distilled water daily for 7 days and served as the control group. Group II was administered an i.p injection of SCO hydrobromide (1.4 mg/kg body weight daily) according to El-Khadragy et al. (2014) for 7 days. Group III was orally administered SSFE (300 mg/kg body weight daily) for 7 days. Group IV was gavaged with SSFE (300 mg/kg body weight daily) for 7 days and 24 h after the last dose; rats in this group received an i.p injection of SCO (1.7 mg/kg body weight daily) for an additional 7 days. Finally, Group V was orally administered N-acetylcysteine (NAC, 100 mg/kg body weight daily) for 7 days and 24 h after the last dose, rats in this group were administered an i.p injection of SCO (1.4 mg/kg body weight daily) for an additional 7 days. All animal were killed 24 h after the last treatment.

Hippocampal homogenates

The hippocampus was removed and washed with ice-cold 50 mM Tris–HCl, pH 7.4. The hippocampus was weighed and homogenized in ice-cold 50 mM Tris–HCl, pH 7.4, to obtain a 10% (*w*/*v*) homogenate. The homogenates were centrifuged at 3000 rpm for 10 min at 4 °C. The supernatants were then utilized for the determination of biochemical parameters. Total protein content was estimated using the standard protocol of Lowry et al. (1951). For the determination of the neurotransmitters, the hippocampus was homogenized in 75% aqueous HPLC grade methanol (10% w/v) and 0.4 mol/L perchloric acid then centrifuged at 4000 rpm for 10 min.

Neurochemical analysis

Hippocampal ACh levels were evaluated using ELISA according to the kit manufacturer's instructions. AChE activity was assayed using the method previously described by Ellman et al. (1961). Norepinephrine (NE), dopamine (DA) and serotonin (5-HT) concentrations in the hippocampus were determined using the method previously described by Pagel et al. (2000). Histamine (HIS) levels were estimated using the protocol previously described by Yoshikawa et al. (2013). Finally, ATP levels were measured using the protocol previously described by Teerlink et al. (1993).

Hippocampal oxidative damage

Thiobarbituric acid-reactive substances (TBARS), markers of lipid peroxidation, were determined in the homogenates as a function of malondialdehyde (MDA) levels to estimate lipid peroxidation in hippocampal tissue according the method described by Janero (1990). Nitrite/nitrate levels and glutathione (GSH) content were assessed using the protocols previously described by Green et al. (1982) and Ellman (1959), respectively.

Hippocampal antioxidant status

The hippocampal homogenate supernatant was used for the determination of the activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-R) according to the methods described by Fisher et al. (2003), Aebi (1984), and De Vega et al. (2002), respectively.

Quantification of hippocampal TNF-α and IL-1β levels

Hippocampal TNF-α and IL-1β (TNF-α; RTA00, R&D Systems and IL-1β; ERIL1B, Thermo Fisher Scientific) levels were determined using ELISA according to the relative kit manufactures instructions.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis

Total RNA was separated from the hippocampus using the RNeasy Plus Mini kit (Qiagen, Valencia, CA, USA). cDNA synthesis was performed using the iScript™ cDNA Synthesis kit (Bio-Rad, CA, USA). Real-time PCR was performed using Power SYBR Green (Life Technologies, CA, USA) on an Applied Biosystems Instrument. The thermal profile for the PCR reaction was 95 °C for 4 min, followed by 40 cycles at 94 °C for 60 s and 55 °C for 60 s. After PCR amplification, the $^{\Delta}$Ct was determined. The PCR primers for the Bax, Bcl-2, iNOS, nuclear factor erythroid 2–related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) genes were synthesized by Jena Bioscience GmbH (Jena, Germany) using the Primer-Blast program from NCBI. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The primer pairs used were previously described by (Almeer and Abdel Moneim 2018).

Histopathological examination

Hippocampal tissue was fixed in 10% neutral buffered formaldehyde for one day, dehydrated in ethyl alcohol, cleared in xylene, and mounted in molten paraffin wax. Sections (4–5 μm) were stained with hematoxylin and eosin and observed using a light microscope (Nikon; Eclipse E200-LED, Tokyo, Japan).

Immunohistochemistry analysis

To investigate apoptosis-related proteins, the prepared hippocampal sections (4-μm thickness) were blocked with 0.1% hydrogen peroxide containing methanol for 15 min to inactivate the endogenous peroxidase. After blocking, the sections were incubated with rabbit polyclonal antibodies against Bax, caspase-3 and Bcl-2 at 4 °C overnight. The sections were rinsed with phosphate-buffered saline and incubated with biotinylated goat anti-rabbit immunoglobulins, followed by incubation with streptavidin-peroxidase complexes at 30 °C for 30 min. The peroxidase activity was developed using 3,3′-diaminobenzidine (DAB). Images were recorded at an original magnification of 400× (Nikon Eclipse E200-LED, Tokyo, Japan).

Statistical analysis

All data are expressed as the mean ± standard error of the mean. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). Duncan's test was used *post-hoc* for comparisons between groups. A P value < 0.05 was considered statistically significant.

Results

Total polyphenolic and flavonoids content in SSFE

Table 1 shows the total polyphenolic and flavonoids contents in SSFE. The total polyphenolic content was 73.6 μg/mg gallic acid equivalent of polyphenols/mL SSFE. Total flavonoids constituent in SSFE was 24.8 μg/mg quercetin equivalents of flavonoids/mL SSFE. According to our findings, a non-significant change was recorded between the initial and final phenolics and flavonoids contents indicating the stability of SSFE throughout the experiment period.

Effect of SSFE on ACh level and acetylcholine esterase AChE activity in hippocampal tissue following SCO-induced memory impairment

Central cholinergic disturbance has been implicated in memory impairment related disorders including dementia. In the present work, treatment with SCO (Group II) increased hippocampal AChE activity significantly ($P < 0.05$) relative to that observed in the control group (Group X). Pretreatment with SSFE (SSFE+SCO treated, Group IV) normalized AChE activity when compared with the SCO-treated group (Group II). Likewise, NAC pretreatment prevented AChE increment following SCO injection. In addition, ACh level was significantly ($P < 0.05$) decreased in the SCO-treated group (Group II) when compared with the control group (Group I). ACh level was significantly increased in the group pretreated with SSFE (Group IV) compared with the SCO-treated group (Group II). These data showed the ability of SSFE to regulate the cholinergic transmission which improves the cognitive function (Fig. 1). Similar findings were also obtained in NAC pretreated group.

Effect of SSFE on NE, DA, 5-HT, HIS and ATP in hippocampal tissue following SCO-induced memory impairment

Cognitive functions are regulated by neurotransmitters. Rats in the SCO-treated group (Group II) had a significant ($P < 0.05$) decrease in NE, DA, 5-HT, HIS, while ATP levels were elevated in the hippocampus. Pre-treatment with SSFE successfully modulated the levels of the assessed neurotransmitters which further confirm its neuroprotective effects (Table 2). NAC was found to restore these neurological mediators to be near the control values.

Effect of SSFE on the oxidative status in hippocampal tissue following SCO-induced memory impairment

Dementia has been associated with over production of reactive oxygen species leading to an imbalance between cellular oxidants and antioxidants. In our experiment, the oxidative status in hippocampal tissue was examined to evaluate the potential antioxidant capacity of SSFE following SCO-induced memory impairment and oxidative reactions. SCO-treated rats (Group II) had a significant ($P < 0.05$) increase in oxidant levels including nitrite/nitrate, and MDA relative to the control group (Group I). One-way ANOVA revealed that the pretreatment with SSFE (Group IV) attenuated the levels of those oxidants compared with the SCO-treated group (Group II). Additionally, the activity of antioxidant defense molecules including GSH, SOD, catalase, GSH-R and GSH-Px was decreased significantly ($P < 0.05$) in the hippocampal homogenate of SCO-treated rats (Group II) when compared to the control group. This cellular antioxidant system was activated significantly in the SSFE+SCO group (Group IV) when

Table 1 Total phenolics and flavonoids content in SSFE

Total phenolic content (mg gallic acid equivalents (GAE)/g SSFE)	73.6 ± 5.8
Total flavonoids content (mg querectin equivalents (QE)/g SSFE)	24.8 ± 3.1

Values are represented as mean ± SD of triplicates

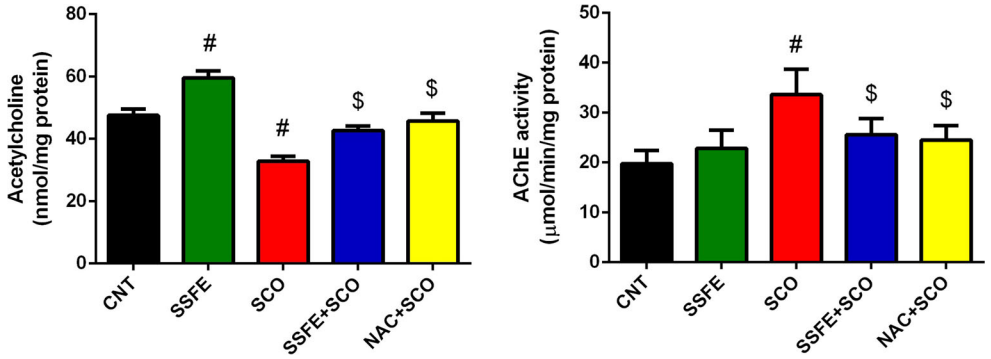

Fig. 1 The effects of pre-treatment with soursop fruit extract (SSFE) on the levels of acetylcholine (ACh) and acetylcholine esterase (AChE) activity in hippocampal tissue of scopolamine (SCO)-injected rats. Data expressed as the mean ± standard error of mean (SEM) ($n = 7$). $^{\#}P < 0.05$, significant change with respect to control group (Group I); $^{\$}P < 0.05$, significant change with respect to SCO-treated group (Group II) using Duncan's *post-hoc* test

compared with the SCO-treated group (Group II) (Figs. 2 and 3). NAC pretreated rats showed a marked decrease in oxidants level and the elevation of antioxidants activity in response to SCO injection.

The protein expression of Nrf2 and HO-1 which play a key role in the expression of different antioxidant enzymes was investigated, to further clarify the underlying molecular antioxidant mechanism of SSFE in SCO-induced memory dysfunction and oxidative stress. SSFE pretreatment effectively upregulated hippocampal mRNA expression of HO-1 and Nrf2 when compared to SCO-treated rats (Fig. 4). This may explain the increase of hippocampal antioxidant defense enzymes. In the same context, pretreated rats with NAC showed the upregulation of HO-1 and Nrf2.

Effect of SSFE on the inflammatory response in hippocampal tissue following SCO-induced memory impairment

Neuroinflammation is an important pathogenic mechanism involved in memory impairment due to neurodegeneration. SCO-treatment caused a marked elevation in hippocampal TNF-α and IL-1β. This was accompanied by upregulation of iNOS protein expression in comparison to the control group. Pretreatment with SSFE for 7 days significantly suppressed these inflammatory mediators and the observed levels were similar to those found in the control group (Fig. 5). NAC pretreatment inhibited the elevation of these chemical messengers.

Effect of SSFE on hippocampal histological alterations following SCO-induced memory impairment

Sections of control rats showed normal hippocampal architecture. However, SCO-treated group showed neuronal degeneration, severe vacuolation, accumulation of inflammatory cells accompanied with blood vessels congestion. SSFE and NAC pretreated groups abolished markedly the histopathological alterations following SCO injection (Fig. 6).

Effect of SSFE on the apoptotic proteins in hippocampal tissue following SCO-induced memory impairment

To investigate the potential anti-apoptotic effect of SSFE in response to SCO injection, we assessed the expression Bcl2,

Table 2 The effects of the pre-administration of soursop fruit extract (SSFE) on serotonin, dopamine, norepinephrine, histamine and adenosine triphosphate content in hippocampus of scopolamine (SCO)-injected rats for 7 days

Parameter	Experimental groups				
	Control	SSFE	SCO	SSFE+SCO	NAC + SCO
Norepinephrine (μg/g tissue)	0.32 ± 0.01	0.47 ± 0.02$^{\#}$	0.18 ± 0.01$^{\#}$	0.22 ± 0.01$^{\#}$	0.30 ± 0.01$^{\$}$
Dopamine (μg/g tissue)	0.97 ± 0.06	1.34 ± 0.05$^{\#}$	0.62 ± 0.02$^{\#}$	0.78 ± 0.04$^{\#\$}$	0.95 ± 0.03$^{\$}$
Serotonin (μg/g tissue)	0.57 ± 0.03	0.68 ± 0.04	0.35 ± 0.01$^{\#}$	0.46 ± 0.02$^{\#\$}$	0.60 ± 0.01$^{\$}$
Histamine (ng/g tissue)	13.85 ± 0.7	16.47 ± 1.1	8.48 ± 0.03$^{\#}$	10.72 ± 0.4	14.08 ± 0.3$^{\$}$
ATP (μmole/g wet tissue)	4.68 ± 0.1	5.13 ± 0.4	7.93 ± 0.2$^{\#}$	6.48 ± 0.2$^{\#\$}$	4.49 ± 0.6$^{\$}$

Data are expressed as the mean ± standard error of mean (SEM) ($n = 7$). $^{\#}p < 0.05$, significant change with respect to control; $^{\$}p < 0.05$, significant change with respect to SCO using Duncan's post-hoc test

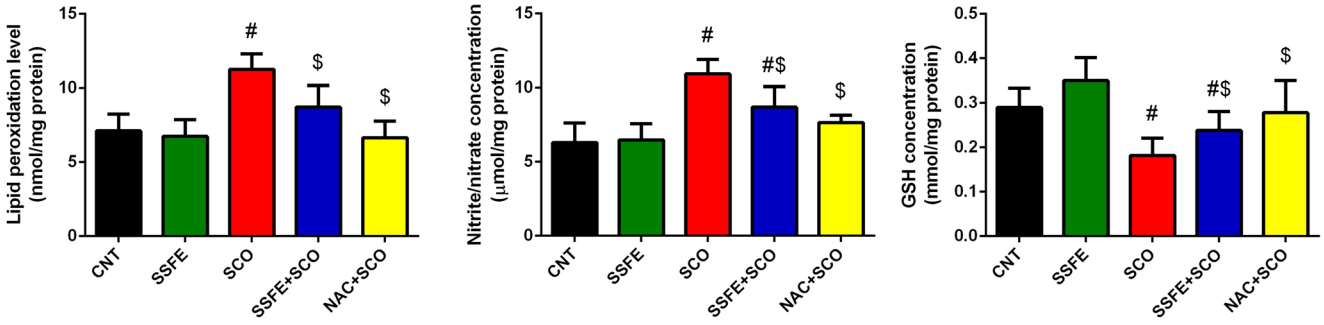

Fig. 2 Ameliorative role of soursop fruit extract (SSFE) pretreatment on lipid peroxidation, nitrites/nitrates and glutathione (GSH) levels in hippocampal tissue of scopolamine (SCO)-treated rats. Data are expressed as the mean ± standard error of mean (SEM) (n = 7). $^{\#}P < 0.05$, significant change with respect to control group (Group I); $^{\$}P < 0.05$, significant change with respect to SCO-treated group (Group II) using Duncan's *post-hoc* test

Bax, and cytochrome *c* in hippocampal tissue using qRT-PCR technique. Our findings demonstrated that SCO-treatment significantly downregulated the expression of Bcl2, an anti-apoptotic protein, upregulated the expression of Bax, pro-apoptotic proteins and increased cytoplasmic cytochrome *c* content when compared with control levels (Fig. 7). Immunohistochemistry method was also employed to confirm the obtained data. SCO-injected rats showed a decrease in Bcl-2 immunoreactivity and an increase in Bax and caspase-3 immunoreactivity in hippocampal tissue. Pre-treatment with SSFE showed mild Bax and caspase-3 immunoreactivity, and a moderate Bcl-2 immunoreactivity, reflecting its anti-apoptotic activity (Fig. 8). The antiapoptotic behavior was also recorded in NAC pretreated rats.

Discussion

SCO-induced amnesia and oxidative reactions in experimental animals have been previously used in the assessment of natural or synthetic anti-amnesic drugs. The current study aimed to investigate the potential anti-amnesic activity of SSFE via modulation of the cholinergic pathway, monoaminergic system, oxidative status, neuroinflammation and apoptotic proteins in hippocampal tissue of rats. The data revealed a disturbance in the central cholinergic system that was evident due to over activation of AChE and decrease in ACh following SCO-treatment. By contrast, pretreatment with SSFE effectively ameliorated alterations in hippocampal cholinergic transmission, reflecting its efficiency in the improvement of cognitive performance.

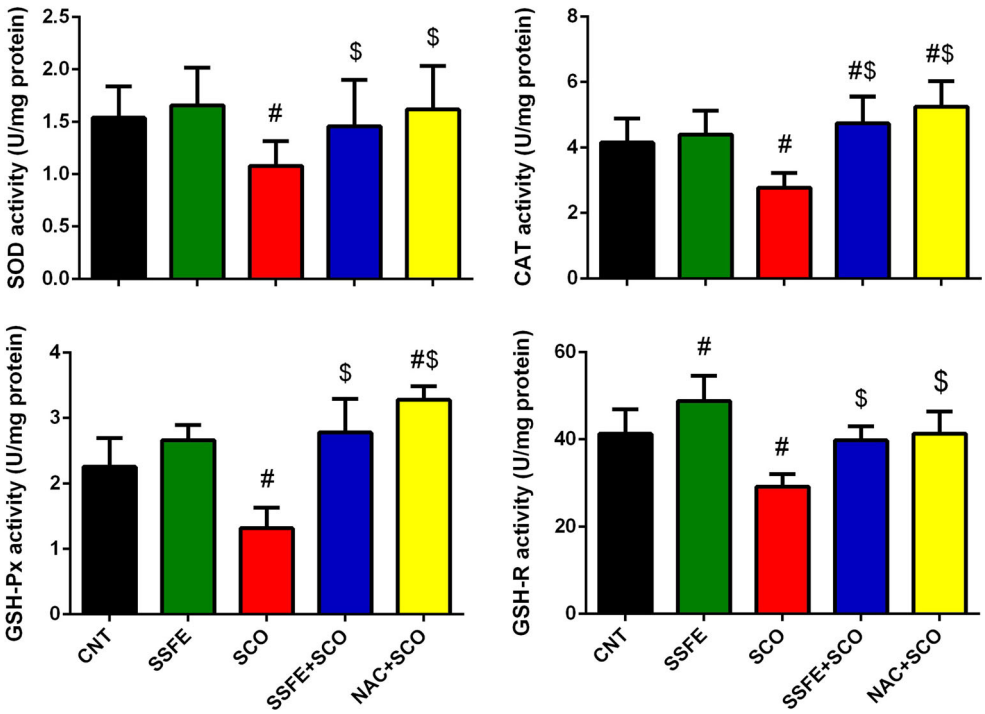

Fig. 3 Ameliorative effects soursop fruit extract (SSFE) pretreatment on the activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-R) in hippocampal tissue of scopolamine (SCO)-treated rats. Data are expressed as the mean ± standard error of mean (SEM) (n = 7). $^{\#}P < 0.05$, significant change with respect to control group (Group I); $^{\$}P < 0.05$, significant change with respect to SCO-treated group (Group II) using Duncan's *post-hoc* test

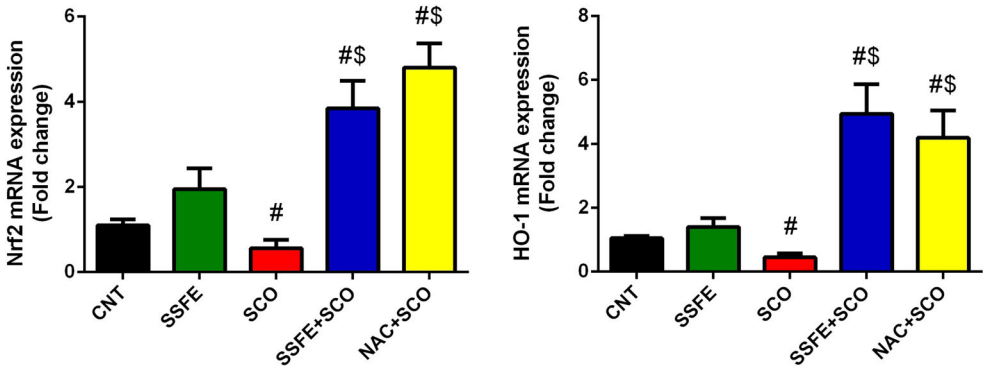

Fig. 4 Ameliorative effects of soursop fruit extract (SSFE) pretreatment on nuclear factor erythroid 2–related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) in hippocampal tissue of scopolamine (SCO)-treated rats. Data are expressed as the mean ± standard error of mean (SEM) (n = 7). $^{\#}P < 0.05$, significant change with respect to control group (Group I); $^{\$}P < 0.05$, significant change with respect to SCO-treated group (Group II) using Duncan's *post-hoc* test

Numerous studies revealed that SCO causes memory and learning disturbances by increasing AChE activity and the muscarinic receptor blockade, leading to a decrease in the levels of ACh. Moreover, SCO may also enhance calcium influx as a result of reactive oxygen species (ROS) over production leading to an increase AChE activity (Kim et al. 2018; Martins et al. 2018). ACh has been characterized as the main neurotransmitter involved in regulating cognitive function. In the hippocampus, degeneration of cholinergic synapses and subsequent cholinergic transmission dysfunction has been associated with AD progression (Moneim 2015). Therapeutic intervention mainly targets enhancement of cholinergic activity through suppression of AChE which degrades ACh into acetate and choline (Adlimoghaddam et al. 2018). Therefore, SSFE may be a candidate for the treatment of memory impairment and other related disorders through its effect on the hippocampal cholinergic system.

The monoaminergic system, HIS and ATP were also disturbed in this study. SCO-treated rats showed a marked decline in 5-HT, NE, DA and HIS, while ATP was elevated in the hippocampus. The neuroprotective effect of SSFE was further confirmed through the regulation of these transmitters. Decreased levels of 5-HT, NE and DA in hippocampal tissue following SCO-treatment was also observed in previous studies (El-Khadragy et al. 2014; Haider et al. 2016). Cognitive processes are maintained and regulated through interactions between various neurotransmitters and their disturbance is associated with AD pathogenesis (Kandimalla and Reddy 2017). 5-HT is the most abundant monoaminergic neurotransmitter in the central nervous system and may be involved in short- and long-term memory and learning processes (Cifariello et al. 2008). In AD, reduced 5-HT and its metabolite, serotonergic receptor dysfunction and the loss of serotonergic neurons impair cognitive performance in humans and in animal models (Ramirez et al. 2014). The decrease in noradrenergic neurons has been linked to the accumulation of Aβ and neurofibrillary abnormalities; both are associated with AD pathogenesis (Heneka et al. 2010). In early AD, NE depletion has been attributed to the inhibition of DA β-hydroxylase, the rate limiting enzyme in NE synthesis from DA, and a reduction in the density of the NE transporter system (Gulyas et al. 2010). The elevation of NE neurotransmission is thought to have a protective role in AD partially through the enhancement of cAMP/protein kinase A signaling pathway and the activation of β-adrenergic receptors (Yang et al. 2012). DA plays a pivotal role in regulating hippocampal synaptic

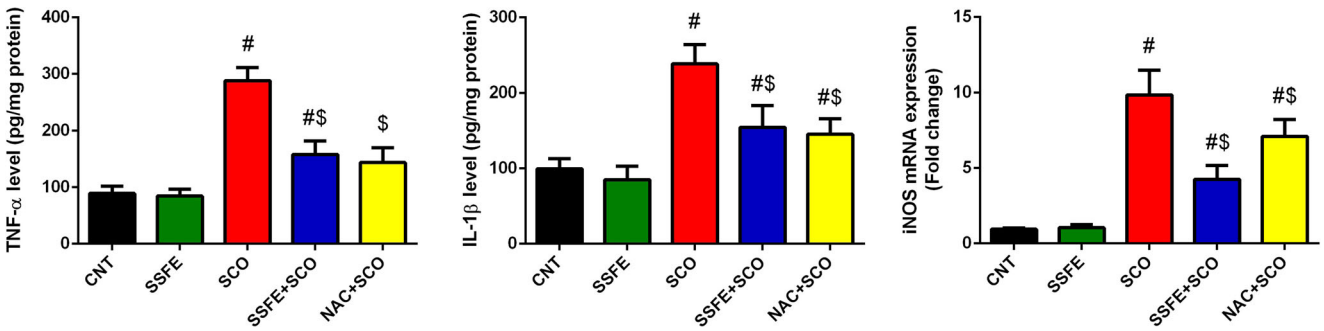

Fig. 5 Ameliorative effects of soursop fruit extract (SSFE) pretreatment on tumor necrosis factor (TNF)-α, interleukin (IL)-1β and nitric oxide synthase (iNOS) in hippocampal tissue of scopolamine (SCO)-treated rats. Data are expressed as the mean ± standard error of mean (SEM) (n = 7). $^{\#}P < 0.05$, significant change with respect to control group (Group I); $^{\$}P < 0.05$, significant change with respect to SCO-treated group (Group II) using Duncan's *post-hoc* test

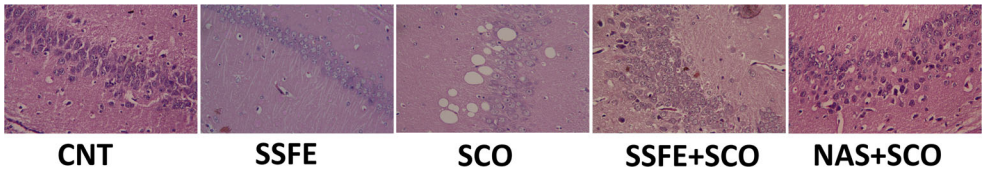

Fig. 6 Effect of soursop fruit extract (SSFE) pretreatment on the hippocampal histological changes caused by SCO injection. (hematoxylin and eosin [H&E], ×400)

plasticity and was also found to be the main determinant of memory coding (Cordella et al. 2018). Amyloid accumulation in brain tissue was reported to be strongly correlated with the degeneration of the dopaminergic system (D'Amelio et al. 2018). Changes in dopaminergic neuron signaling including decreased DA concentration and its receptors have been identified in AD and are linked to cognitive dysfunctions in humans and in experimental animal models. The decrease in DA level is due in part to the suppression of tyrosine hydroxylase (D'Amelio et al. 2018). In the central nervous system, HIS regulates different physiological functions including learning and memory integration (Baronio et al. 2014). Histaminergic system abnormalities have been identified in neurodegenerative disorders including AD. Several postmortem and experimental investigations revealed a decrease in HIS in different brain regions due to the reduction of histaminergic receptor expression (Zlomuzica et al. 2016). However, the findings regarding HIS in AD remain controversial. Change in the activity of monoamine oxidase may contribute to the pathogenesis of neurodegenerative conditions including AD and Parkinson's disease. The reduction in monoamines following SCO exposure may be due to an increase in the activity of monoamine oxidase which breaks down these neurotransmitters in the synaptic cleft (Ogunsuyi et al. 2018). ATP is a neuromodulator released from glial cells and neurons in the peripheral and central nervous system. Over activation of this purinergic molecule and its receptors has been associated with cognitive deficits (Koch et al. 2015). A previous study revealed that β-amyloid increases ATP leakage and activates P2X receptors which enhances excitatory synaptic activity (Saez-Orellana et al. 2016).

The potential neuroprotective effect of *A. muricata* extracts and its biological active ingredients have received a little attention. However, one study found that pretreatment with an ethanolic extract of *A. muricata* (100 mg/kg) stembark for 16 consecutive days increased the levels of 5-HT, DA and NE following cold immobilization-induced oxidative stress, indicating its adaptogenic potential (Padma et al. 2001). Another study showed that treatment with an ethanolic extract of *A. muricata* (50, 150 and 300 mg/kg) produced both sedative and antidepressant effects in rats (Bikomo et al. 2017).

Flavonoids have been identified as a major constituent of SOP and many other herbs, fruits and vegetables (Sandoval et al. 2018). Previous studies showed that flavonoids may enhance learning and memory ability in different animal models via inhibition of AChE activity and antioxidant capacity (Kim et al. 2009; Pattanashetti et al. 2017). Alkaloid contents in Annonaceae species appear to modulate changes in AChE activity (Lee et al. 2015). It has been demonstrated that plant-derived alkaloids may reverse the disturbances in cognitive processes due to their nitrogen-containing structures (Pereira et al. 2010). The binding sites of AChE involve the interaction of the positively-charged nitrogen allowing inhibition by non-alkaloid compounds, mainly terpenes, xanthones and coumarins (Houghton et al. 2006). Therefore, the rich phytcohemical constituent in SSFE may be responsible for the modulation of neurochemical alterations in SOC-induced amnesia.

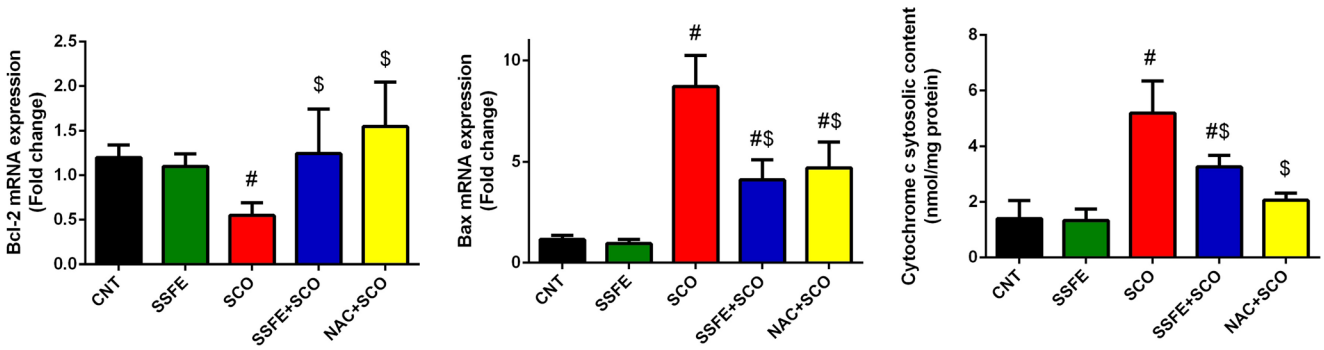

Fig. 7 Mitigation effects of soursop fruit extract (SSFE) pretreatment on the mRNA expression of apoptotic proteins (Bcl-2 and Bax) and cytochrome *c* content in hippocampal tissue of scopolamine (SCO)-treated rats. Data of mRNA levels for Bcl-2 and Bax (mean ± standard error of mean [SEM] of 3 assays) were normalized to *GAPDH* mRNA level and are shown as fold induction (in log2 scale) relative to the mRNA level in the control group. $^{*}P < 0.05$, significant change with respect to control group; $^{**}P < 0.05$, significant change with respect to SCO-treated group using Duncan's *post-hoc* test

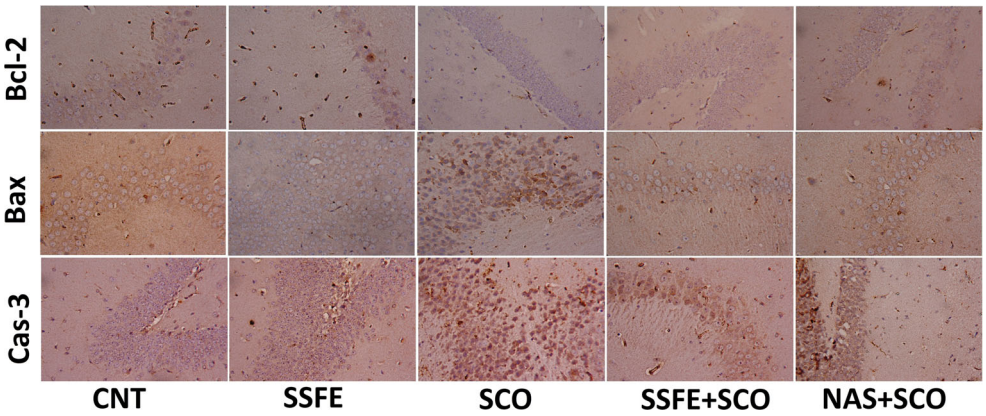

Fig. 8 Immunohistochemical study of Bcl-2, Bax and caspase-3 in hippocampal tissue of different treated groups following SSFE and/or SCO treatment. (×400)

Several experimental studies revealed the involvement of oxidative stress in the pathogenesis of neurological disorders including AD. The brain has poor antioxidant capacity due to high oxygen utilization and polyunsaturated fat content. In the present study, SOP-induced imbalance between the oxidants and antioxidants in hippocampal tissue was evident due to increased levels of lipid peroxidation, nitrites/nitrates, and the depletion of GSH, SOD, catalase, GSH-R and GSH-Px. Moreover, the altered oxidative profile was highlighted by disturbances in the HO-1 and Nrf2 pathways. SSFE was able to restore the balance between the cellular oxidants and antioxidants, demonstrating its neuroprotective efficiency in oxidative stress-induced cognitive dysfunction following SCO-treatment. This action may be due to its high polyphenolic constituent. Lipid peroxidation has been identified as a major characteristic in neurodegenerative diseases including AD. The elevated level of lipid peroxidation in brain tissue is due to the generation of a superoxide anion following mitochondrial dysfunction which then suppresses antioxidant enzymes. Moreover, free radicals attacks the brain polyunsaturated fatty acids and enhances lipid peroxide formation (Huang et al. 2016). Our findings demonstrated that the increase in nitrite/nitrate level is due to the over expression of iNOS which considered the rate limiting enzyme in nitric oxide synthesis. Nitric oxide interacts with the superoxide anion forming peroxynitrite which is a powerful oxidant that causes neuronal cell death (Al Omairi et al. 2018). The ROS and their derivatives generated following SCO-treatment were found to interact with cellular macromolecules and suppressed the antioxidant defense system including GSH, SOD, catalase and GSH-Px (El-Khadragy et al. 2014). The induction of oxidative damage enhances the translocation of Nrf2 from the cytosol to the nucleus which activates the expression of various detoxifying and antioxidant enzymes. The Nrf2 pathway is likely dysfunctional in hippocampal neurons of AD. It has been reported that Nrf2 does not translocate into the hippocampal nuclei in AD which affects the function of the antioxidant defense system (Ramsey et al. 2007). This may explain the downregulation of Nrf2 following SCO-treatment. In addition, antagonism of muscarinic receptors has been found to suppress Nrf2 expression which regulates the expression of antioxidant defense enzymes (Venkatesan et al. 2016). HO-1 represents the major downstream enzymes to Nrf2, and mainly catalyzes heme catabolism into biliverdin, free iron, and carbon monoxide. Biliverdin reductase converts biliverdin into bilirubin which is considered an intracellular antioxidant (Ryter and Tyrrell 2000). In recent studies, the activity of HO-1 in AD experimental models was reported to be decreased (Morroni et al. 2018). The authors attributed this decrease to the downregulation of Nrf2 expression in the brain tissue which is in consistent with our findings.

Researchers suggest that medicinal plants may protect and prevent different disorders associated with oxidative stress such as cancer, cardiovascular and neurodegenerative diseases. Phenolic constituents of herbs have the ability to scavenge ROS and chelate metals involved in lipid peroxidation (Odubanjo et al. 2018). The possible mechanism for this action may involve the hydroxyl groups of polyphenols, which have been shown to form hydrogen bonding with the polar head of phospholipids on the membrane surface (Manach et al. 2004). This adsorption of polyphenols probably limits the access of aqueous oxidants to the membrane surface and their initial attack on that surface, thereby reducing oxidative damage and lipid peroxidation (Schrag et al. 2013). The antioxidant capacity of *A. muricata* has been demonstrated in different experimental models. SCO showed potent antioxidant properties in fibroblastic cultures treated with hydrogen peroxide through scavenging ROS and the inhibition of NADPH oxidase enzyme; the main sources of free radicals (Zamudio-Cuevas et al. 2014). In addition, the ethyl acetate extract of *A. muricata* leaves decreased the level of lipid peroxidation and increased GSH, catalase and SOD activities in ethanol-induced oxidative stress and gastric damage (Moghadamtousi et al. 2014). The activation of Nrf2-mediated antioxidant enzymes including HO-1 was shown to protect neurons from oxidative damage in different experimental models (Seo et al. 2018; Zhang et al. 2014). The over expression of Nrf2 and HO-1 reflects the antioxidant response produced by SSFE following SCO-treatment.

Inflammation is one of the mechanisms involved in the development of AD and its associated poor cognitive performance (Yin et al. 2015). This study revealed increased concentrations of TNF-α and IL-1β following SCO-treatment. Over production of pro-inflammatory cytokines including TNF-α and IL-1β may accelerate neurodegeneration and affect memory function. Elevation in the levels of these inflammatory mediators may be due to the activation of the NF-κB pathway (Zhu et al. 2016). Increased concentrations TNF-α and IL-1β are thought to affect memory function by blocking the cholinergic system and promoting the accumulation of β-amyloid protein that enhances neuronal death (He et al. 2007). SSFE demonstrated anti-inflammatory activity by decreasing TNF-α and IL-1β in hippocampal tissue. The anti-inflammatory properties of *A. muricata* extracts have been previously studied. SCO decreased the levels of TNF-α, IL-1β, IL-6, and nitric oxide in lipopolysaccharide treated Raw 264.7 cells (Laksmitawati et al. 2016). These pro-inflammatory cytokines were also decreased in a rat model of Freund's adjuvant-induced arthritis following treatment with the ethanolic extract of *A. muricata* (Chan et al. 2010). *A. muricata* was reported to possess both anti-inflammatory and analgesic effects via the interaction with the opioidergic pathway and suppression of inflammatory mediators (Ishola et al. 2014).

Neuronal apoptosis is positively correlated with memory impairment through the suppression of anti-apoptotic proteins and activation of pro-apoptotic proteins (Kale et al. 2018). We found that SCO-treatment triggered an apoptotic cascade that was evident through the upregulation of Bax and caspase-3 proteins expression, elevation of systolic cytochrome *c* content and downregulation of Bcl-2 protein expression. Previous reports attributed the increase of Bax and caspase-3, cytochrome *c* and the decrease of Bcl-2 expression in neuronal tissue of AD models to the β-amyloid deposition which disturbs mitochondrial dysfunction and calcium homeostasis, and enhances ROS production (Hu et al. 2018; Jiao et al. 2018; Yoo et al. 2017). However, SSFE pretreatment may substantially alleviate the alterations in the apoptotic proteins, suggesting a potential neuroprotective effect of SSFE in oxidative stress-induced hippocampal apoptosis following SCO exposure. This may be due to the activation of the Nrf2 defense system which was found to attenuate D-galactose induced neuronal apoptosis and memory dysfunction in an AD model (Dong et al. 2017).

Conclusion

The findings from this study suggest that SSFE may have a neuroprotective effect in SCO-induced neurochemical alterations, oxidative damage, neuroinflammation and neuronal apoptosis in hippocampal tissue. This may be in part, due to, maintaining cholinergic, monoaminergic and purinergic transmission, enhancing cellular GSH antioxidant enzymes via activation of Nrf2/HO-1 pathway, anti-inflammatory activity through the modulation of pro-inflammatory cytokines, and anti-apoptotic activity through enhancing anti-apoptotic and suppressing pro-apoptotic proteins. Collectively, our findings revealed that SSFE should be further investigated as a potential alternative anti-amnesic agent in SCO-induced memory deficit.

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Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

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