Saudi Journal of Biological Sciences 27 (2020) 2829–2838

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

HPLC-PDA-MS/MS profiling of secondary metabolites from *Opuntia ficus-indica* cladode, peel and fruit pulp extracts and their antioxidant, neuroprotective effect in rats with aluminum chloride induced neurotoxicity

Seham S. El-Hawary [a], Mansour Sobeh [b,f,*], Wafaa K. Badr [c], Mohamed A.O. Abdelfattah [d], Zeinab Y. Ali [e], Mona E. El-Tantawy [c], Mohamed A. Rabeh [a], Michael Wink [b]

[a] Department of Pharmacogosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt
[b] Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Im Neuenheimer Feld 364, 69120-Heidelberg, Germany
[c] Department of Medicinal plants and natural products, National Organization of Drug Control and Research, Giza, Egypt
[d] College of Engineering and Technology, American University of the Middle East, Kuwait
[e] Department of Biochemistry, National Organization of Drug Control and Research, 12553 Giza, Egypt
[f] AgroBiosciences Research Division, Mohammed VI Polytechnic University, Lot 660–Hay Moulay Rachid, 43150 Ben-Guerir, Morocco

ARTICLE INFO

Article history:
Received 8 December 2019
Revised 30 June 2020
Accepted 2 July 2020
Available online 9 July 2020

Keywords:
Opuntia ficus-indica
HPLC-MS/MS
Neurotoxicity
$AlCl_3$
In vivo
Molecular docking

ABSTRACT

Opuntia ficus-indica (L.) Mill. (OFI), also known as Indian fig *Opuntia* or prickly pear, is a member of the family Cactaceae that produces edible, nutritionally rich sweet fruits. It has been traditionally used to treat several health disorders and is considered to possess various therapeutic properties. In this work, we have characterized 37 secondary metabolites using HPLC-MS/MS, identified the polysaccharide from the fruits and cladodes pulp, and estimated the neuroprotective activity. All tested extracts exhibited substantial antioxidant activities *in-vitro* and neuroprotective potential in $AlCl_3$ induced Alzheimer's condition. Administration of OFI extracts attenuated $AlCl_3$ induced learning and memory impairment as confirmed from passive avoidance test and counteracted the oxidative stress as manifested from decreasein the elevated MDA level, increased TAC, GSH, SOD and CAT levels. OFI extracts significantly decreased the elevated brain levels of proinflammatory cytokines (NF-κβ and TNF-α), increased anti-inflammatory cytokine (IL-10), and monoamine neurotransmitters (NE, DA, 5-HT) as compared to positive control group. The extracts showed a significant decrease in acetylcholinesterase level (AChE) as compared with $AlCl_3$. Furthermore, molecular docking was performed to investigate the ability of the major constituents of OFI extracts to interact with acetylcholinesterase (AChE) and serotonin transporter (SERT). Among the tested extracts, cladodes contain highest phenolic content and exhibited the highest antioxidant, anti-inflammatory and neuroprotective activities, which could be attributed to presence of several polyphenols, which could function as AChE and SERT inhibitors. *Opuntia ficus-indica* might be promising candidate for treating Alzheimer disease, which makes it a subject for more detailed studies.

1. Introduction

Formation and inactivation of reactive oxygen species (ROS) is a common biological feature in all aerobic organisms. Despite being required for normal cell function and immune response, high concentrations of ROS lead to oxidative stress, which can damage DNA (induce mutations), proteins and bio membranes. Several diseases and health conditions in humans are linked to oxidative stress including cancer, inflammation, diabetes mellitus, ischemia, pulmonary fibrosis, aging and Alzheimer's disease (AD). The latter represents a neurodegenerative disorder leading to progressive

* Corresponding author at: AgroBiosciences Research Division, Mohammed VI Polytechnic University, Lot 660–Hay Moulay Rachid, 43150 Ben-Guerir, Morocco.
E-mail address: mansour.sobeh@um6p.ma (M. Sobeh).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.07.003
1319-562X/

dementia among the elderly (Feldman and Quenzer, 1984; Dumont and Beal, 2011; Van Wyk and Wink, 2017).

Natural antioxidants that are abundant in several foods and medicinal plants have a remarkable free radical scavenging potential that could be useful to counteract Alzheimer's disease and other neurological disorders related to oxidative stress (Chaouch et al., 2016a, Van Wyk and Wink, 2017, 2015; El-Hawary et al., 2018, 2016; El Tanbouly et al., 2017).

Opuntia ficus-indica (L.) Mill., known as Indian fig *Opuntia,* barbary fig, cactus pear, spineless cactus, and prickly pear, is a member of the family Cactaceae. This cactus has a Mexican origin but is now widely distributed in arid and semi-arid regions of the New and Old World. It produces edible, nutritionally rich sweet fruits and its spathulate stems (called cladodes) are ingredients of Mexican cuisine. Fruits and young stems are traditionally utilized to treat hypertension, asthma, edema, diabetes, burns, indigestion, and other health disorders (Chaouch et al., 2016b; Galati et al., 2003; Barba et al., 2017). They also exhibit antioxidant, anticancer, anti-inflammatory and anti-allergic properties (Barba et al., 2017; Ammar et al., 2015; Benayad et al., 2014). Flowers were reported to possess antioxidant, antimicrobial, anti-ulcer, anti-inflammatory and wound healing activities, which were attributed to isorhamnetin, quercetin and kaempferol glycosides.

The fruits contain phenolic alkaloids (betalains) such as betacyanin and betaxanthin, and glycosylated flavonoids that exhibit several pharmacological activities. The red and yellow-colored betalains which are water-soluble pigments are used as natural food colorant (Stintzing et al., 2005; Ahmed et al., 2005). In terms of antioxidant potential, cactus fruits are twice as potent as pears, apples, tomatoes, bananas, white grapes, and almost equipotent to red grapes and grapefruit (Ahmed et al., 2005; Stintzing and Carle, 2006).

In the present study, we have characterized the polyphenolic constituents of extracts from *O. ficus-indica* cladodes, fruit peel and fruit pulp and investigated their antioxidant and neuroprotective activities.

2. Materials and methods

2.1. Drugs and chemicals

Aluminum chloride ($AlCl_3{\cdot}6H_2O$) from NODCAR (Giza, Egypt), donepezil reference drug from Eva pharma (Cairo, Egypt) (Donazil tablets), DPPH from Alfa Aesar Co., (Karlsruhe, Germany), L-noradrenaline from MP Biomedicals Co., (Eschwege, Germany), dopamine hydrochloride from Sigma Aldrich Co. (Steinheim, Germany), and serotonin hydrochloride from Alfa Aesar Co., (Karlsruhe, Germany) were used. The used neurotransmitters are of HPLC grade. Folin-ciocalteu reagent for phenolic content obtained from LOBA Chemie PVT., Ltd, India. All other used materials were of highest grade, commercially available chemicals and solvents.

2.2. Plant material and extraction

Plant samples (5 kg) of *Opuntia ficus-indica* (cladode peel and fruits) were collected from Orman Botanical garden, Giza, Egypt on July 2016. The plant was authenticated by Eng. Trease Labib Yousef (National Gene Bank and Orman Botanical Garden Consultant). The samples were extracted with 100% methanol (3 × 2 L) at ambient temperature. The extracts were combined, filtered and reduced under vacuum at 40 °C. After freezing at −70 °C, the extracts were lyophilized yielding fine dried powder.

2.3. Preparation and purification of the polysaccharides

The polysaccharides of both fruits and cladodes were prepared according to Laidlaw and Percival (1950). Sliced deseeded fruits and cladode pulps (100 g) were extracted with hot water (3 × 500 ml at 90–95 °C) for 12 h until complete extraction of the polysaccharides was reached (tested by giving no cloudiness or precipitate on addition of four volumes of absolute alcohol to one volume of the extract). Polysaccharides were precipitated by addition of four volumes of absolute alcohol to one volume of each extract, centrifuged at 10,000 rpm for 15 min and filtered. The obtained polysaccharides were purified according to White and Rao (1953). They were separately re-dissolved in 50 ml water and the solutions were re-precipitated with 200 ml ethanol. The products were filtered, washed successively with ethanol and ether and then dried in vacuum desiccators over anhydrous calcium chloride to give (2 g) of fruits pulp mucilage representing 2% of the dried weight and (1.87 g) of cladode pulp representing 1.87% of the dried weight.

2.4. Hydrolysis of the polysacchrides

The purified polysaccharides were hydrolyzed according to Pazur et al. (1986). Each polysaccharide (100 mg) was separately acid hydrolyzed by refluxing with 5 ml of 2 M HCl for 2 h in a boiling water bath. After hydrolysis, a slightly dark flocculent precipitate was obtained in each case and was filtered off. Excess hydrochloric acid was removed from the filtrate by neutralization with sodium carbonate solution. The extracts were filtered and evaporated under reduced pressure.

2.5. Chromatographic investigation of the polysaccharides hydrolysate

HPLC analysis was carried out using Agilent chromatographic system (series 1200) coupled to refractive index detector and equipped with quaternary pump, degasser along with auto injector. The chromatographic data was acquired by Agilent software. Samples obtained were analyzed by an Aminex-carbohydrate HPX-87 column (L 250 mm × ID 4.6 mm, particle size (5 μm)) under isocratic elution with water, injection volume 20 μL. The flow rate was set to 0.5 ml/min and the temperature was kept at 85 °C and 50 °C for the column and the detector respectively. The qualitative identification was achieved by comparing relative retention times of the individual sugars to those of authentic sugars.

2.6. HPLC-PDA-Ms/Ms

The phytochemical constituents in the cladodes, fruit peel and pulp extracts were investigated using a ThermoFinnigan LCQ-Duo ion trap mass spectrometer (ThermoElectron Corporation, Waltham, Ma, USA) with ESI source (ThermoQuest Corporation, Austin, Tx, USA) (Sobeh et al., 2017a). A Discovery HS F5 column (15 cm × 4.6 mm ID, 5 μm particles, Sigma-Aldrich Co Steinheim, Germany) was employed with a ThermoFinnigan HPLC system. The mobile phase was made of water and acetonitrile (ACN) (Sigma-Aldrich GmbH, Steinheim, Germany) (0.1% formic acid each). ACN was 5% at 0 min, then increased to 30% over 60 min, and to 90% within the last 30 min. The column was then reconditioned for 10 min to the initial conditions with (5% ACN). The flow rate was maintained at 1 ml/min with a 1:1 split before the ESI source. The extracts (25 μL) were injected using autosampler surveyor ThermoQuest. Xcalibur software (Xcalibur™ 2.0.7, Thermo Fischer Scientific, Waltham, Ma, USA) controlled the machine. The MS operated in the negative mode as previously reported (Sobeh et al., 2017b).

2.6.1. In vitro antioxidant activity evaluation

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging potential of the extracts was determined as previously described by (Ghareeb et al., 2018).

2.6.2. Estimation of total phenolic content colorimetrically

Total polyphenols content (TP) of the different parts were determined following the procedure described by (Kumazawa et al., 2002) and the procedure reported in the (European Pharmacopoeia 4th edition 2002) using Folin-Ciocalteu colorimetric method. The TP content was expressed as gallic acid (mg) equivalent (GAE)/g of the dry plant material using standard calibration curve.

2.7. Biological evaluation

2.7.1. Acute oral toxicity test

The acute toxicity test for cladode, peel, fruit pulp and polysaccharide extracted from *Opuntia ficus-indica* was conducted following the guidelines of the Economic Co-operation and Development (OECD) organization no. 423, a step-wise procedure with 3 animals of single sex per step (OECD, 2001). A single daily dose of extracts 5, 50, 300, 2000 mg/kg body weight was administered orally, for 14 days. All animals were monitored daily for any sign of toxicity or mortality (OECD, 2001).

2.7.2. Experimental design

Fifty-six adult male Sprague Dawley rats of 180–200 g weight were used. Animals were obtained from the animal house of National Organization for Drug Control and Research, Giza, Egypt. Rats were housed in stainless steel cages and maintained under controlled temperature (25 ± 2 °C), humidity (55 ± 5%) and light (12 h on/12 h off) throughout the experiment. They obtained a standard basal diet with free access to water. Rats were acclimatized for one week before the start of the experiment. In order to evaluate the health status, body weight measurement, examination of physical appearance, and deviation from normal behavioral parameters were performed on daily basis. Furthermore, water and food intake were measured daily. No signs of abnormal symptoms were observed during the experimental time. No toxic signs/mortality were observed over the experiment time. The study was conducted in accordance with the ethical guidelines of Faculty of Pharmacy, Cairo University, and performed according to guidelines of the US National Institutes of Health on animal care (approval number: MP1505). The ethics committee Institutional Animal Care and Use Committee (IACUC) (Cairo University) specifically approved the current study (CU/III/F/29/16).

The animals were divided into 7 groups, 8 rats each. Group1 received distilled water and served as control. Group 2 received a daily dose of $AlCl_3$ (70 mg/kg, i.p.) freshly dissolved in distilled water for six weeks (Ali et al., 2016). Group 3 received donepezil (an inhibitor of acetylcholinesterase) at a daily oral dose by gavage of 10 mg/kg body weight (b.w.) (Shin et al., 2018) with daily dose of $AlCl_3$ (70 mg/kg, ip). Groups 4 to 7 received a daily oral dose of extracts (cladodes, fruit peel, fruit pulp and polysaccharide) (100 mg/kg b.w.) freshly dissolved in distilled water with daily dose of $AlCl_3$ (70 mg/kg, ip). The passive avoidance test was conducted at the end of the experiment to evaluate the learning and memory functions of the experimental rats. For other biological markers, blood samples were collected under light anesthesia with ether from the *retro*-orbital venous plexus after 6 weeks. The samples were then centrifuged at 3000 rpm for 10 min to obtain the serum and kept frozen for determination of total antioxidant capacity (TAC). Rats were sacrificed by decapitation under mild ether anesthesia and then their brain tissues were dissected, washed with ice-cold saline and were either subjected immediately for analysis or kept frozen at – 80 °C. The brain was homogenized in 20 mM potassium phosphate buffer pH 2.5 to give 20% brain homogenate (W/V) and centrifuged at 3000 rpm for 10 min. For the determination of monoamine neurotransmitters, frozen brain was homogenized in 75% aqueous methanol. The supernatants were used to estimate the concentrations of catecholamine [norepinephrine (NE), dopamine (DA) and serotonin (5-HT)], acetylcholinesterase (AChE), oxidative stress markers (SOD, CAT, GSH), lipid peroxides expressed as malondialdhyde (MDA) and the inflammatory mediators (NF-κβ, IL-10 and TNF-α).

2.7.3. Assessment of learning and memory functions

Learning and memory functions in rats were determined by the passive avoidance task as behavioral model. The apparatus composed of light and dark chambers with a metal grid floor. Both chambers are separated by a wall that has a door. The animals were tested on two consecutive days. In the acquirement trial, each rat was individually placed in the light chamber. Rapidly after being in the dark chamber, an electric shock (40 V, 0.5 Ma for 1 s) was delivered to the animal's feet through the grid. The animal was directly taken out from the apparatus and returned back to the cage. After 24 h the rat was placed in the light chamber again and the time taken to enter the dark chamber was recorded as step-through latency. If the animal did not enter the dark chamber within a 3-min test period, the test was terminated and the step-through latency was noted as 180 sec (Prema et al., 2016).

2.7.4. Determination of brain monoamine neurotransmitter levels

Brain homogenate was prepared using electric homogenizer in which one gram of the rat brain was homogenized using 5 ml of 75% aqueous methanol then centrifuged for 5 min at 9000 rpm, then the supernatant was filtered using Millex® syringe filter unit (pore size 0.22 μm), Sigma Aldrich, Germany. Determination of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in brain homogenates was done using HPLC. A reversed phase column (Chromasil ODS C18, 150 × 4.6 ID, 5 μm) was used. A 20 mM potassium phosphate solution, pH 2.5 was used as mobile phase. The flow rate was 1.5 ml/min and the compounds were detected using a UV detector at 210 nm. NE, DA, 5-HT concentrations were quantified using HPLC and a calibration curve was constructed using external standard as described in (Ahmed et al., 2016).

2.7.5. Estimation of acetylcholinesterase (AChE) activity

In the brain tissue homogenate, AChE content was measured according to the manufacturer's instructions by using Kits from Biodiagnostic company, Giza, Egypt. The assay is based on measurement of the change in absorbance at 405 nm following the increase of yellow color produced from thiocholine reacting with dithiobisnitrobenzoate ion (DTNB reagent).

2.7.6. Estimation of oxidative stress markers

Markers of oxidative stress in both serum and brain homogenates were determined by following the instructions of manufacture of kits (Biodiagnostic Company, Giza, Egypt). The total antioxidant capacity (TAC) was quantified in the serum, SOD, lipid peroxidation, catalase (CAT) GSH content were quantified in the brain homogenates colorimetrically using standard calibration curves as described in the manufacture instructions.

2.7.7. Estimation of neuro-inflammation markers

For the determination of the inflammation markers in brain homogenates, Enzyme-linked Immune Sorbent Assay (ELISA) technique was used to determine the levels of tumor necrosis factor alpha (TNF-α) and Interleukin-10 (IL-10), using Quantikine kit (R&D Systems, USA). Nuclear factor-kappa B (NF-κB) was deter-

mined using Biosource kit (California, USA) as described in the manufacture instructions.

2.8. Molecular modeling

To gain information about the molecular mechanism by which the studied plant extracts work to enhance learning and memory functions, some of the abundant secondary metabolites in the extracts were docked into two proteins that are renowned to be relevant in (AD) prognosis and cognition functions, namely the acetylcholinesterase and the serotonin transporter protein. The structures of the selected compounds were drawn using the builder tool of the software molecular operating environment (MOE), 2013.08; Chemical Computing Group Inc., Montreal, QC, Canada, H3A 2R7, 2016. The ionized form of the selected compounds was adjusted using the wash option, and then had their energy minimized using the default force-field mmff94x. The structures of the crystallized recombinant human acetylcholinesterase, PDB id: 4EY7 and human serotonin transporter, PDB id: 5I6X altogether with the corresponding bound ligands were downloaded from protein data bank (www.pdb.org). Preparing the proteins for docking was done by assigning the protonation state to add the missed hydrogen atoms, geometry, and energy minimization. The default docking protocol that applies triangle matcher placement method and London dG scoring function was adopted for docking the extracts' compounds to the binding sites of the aforementioned proteins retaining 20 poses. The docking poses were refined by force-field energy minimization applying London dG scoring function (Sobeh et al., 2017a).

2.9. Statistical analysis

The results were expressed as mean ± SE and analyzed by one-way ANOVA using SPSS (version 22). Significant differences between the groups were assessed by Duncan's new multiple-range test. The level of $p < 0.05$ was considered significant.

3. Results

3.1. Chemical constituents of Opuntia ficus-indica (OFI) extracts

The chemical constituents of extracts from different plant parts of *Opuntia ficus-indica* were characterized using HPLC-MS/MS (Fig. 1 and Table 1). In total, 37 secondary metabolites were characterized based on retention times, molecular weight, fragmentation pattern, and comparisons with reported data from the plant and authentic compounds. The extracts were rich in phenolic acids, flavonoids and fatty acids.

A series of *O*-methylated flavonoids dominated the extract. They were detected at $[M-H]^-$ *m/z* 785, 769, 755, 623, and 477 and a daughter ion at *m/z* 315 and assigned to isorhamnetin glucosyl-rutinoside, isorhamnetin rhamnosyl-rutinoside, isorhamnetin pentosyl-rutinoside, isorhamnetin rutinoside, and isorhamnetin glucoside, respectively (Table 1 and Fig. S1), along with the aglycone isorhamnetin (Benayad et al., 2014).

Several phenolic acids were detected, among them sinapic acid hexoside at $[M-H]^-$ *m/z* 385, eucomic acid at $[M-H]^-$ *m/z* 239, and piscidic acid at $[M - H]^-$ *m/z* 255. Also, a 7-glucosyl-oxy-5-methyl flavone glucoside was tentatively identified at $[M-H]^-$ *m/z* 611 and two daughter ions at 251 and 431 (Table 1 and Fig. S1). However, no betalains were detected in the extracts and this might be attributed to their lower concentration.

3.2. Polysaccharide constituents of Opuntia ficus-indica extracts

Polysaccharides were extracted from the fruits and cladodes pulp. After hydrolysis we detected six sugars by HPLC. Data are shown in Table 2.

3.2.1. In vitro antioxidant evaluation

The methanol extracts of the different plant parts displayed *in vitro* radical scavenging activity; however, the activity of the cladodes surpassed the other extracts followed by that of the peel then the pulp and finally the polysaccharides, (Table 3). Similar activities were reported from Italian *Opuntia ficus-indica* cladodes and others (Petruk et al., 2017).

3.2.2. Estimation of phenolic content

The methanol extract of cladodes contained higher content of phenolic compound (236.5 mg (GAE)/g dry wt) followed by fruits peel extract (165.2 mg (GAE)/g dry wt) and finally fruits pulp extract (53.48 mg (GAE)/g dry wt). The higher polyphenol content in cladodes extract than the fruits peel and fruits pulp extracts may explain the higher activity of cladodes compared to that of fruits peel and pulp in the pharmacological studies (antioxidant, anti-inflammatory activity and neuroprotective activity).

3.3. Acute oral toxicity test

The obtained result showed that the oral administration of cladode, peel, fruit and polysaccharide extracts were non toxic and safe up to 2000 mg/kg body weight for 14 days. Therefore, one-twentieth of this tolerated dose (i.e 100 mg/kg body weight/day) was chosen for comparative evaluation of the possible neuroprotective activity of cladode, peel, fruit and polysaccharide extracts.

3.4. Effect of the different extracts on oxidative stress marker levels in vivo

In rats, administration of $AlCl_3$ for six weeks significantly reduced the serum total antioxidant capacity (TAC), superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) and increased malondialdhyde (MDA) levels when compared to untreated control group. Rats treated with the extracts demonstrated a significant increase in TAC, SOD, GSH and CAT levels and decreased the elevated MDA level when compared to the AD rats. The cladodes extract showed the highest activities followed by the peel then the pulp extracts and finally the cladode polysaccharides (Fig. 2).

3.5. Effect of the extracts on learning and memory functions

In the passive avoidance task, an animal ought to learn to escape or avoid the electric shock exposure in the dark chamber. All the nocturnal animals including rats only choose, by nature, a dark environment, but the animal must suppress such tendency by remembering the electric shock. All the tested extracts improved memory and significantly increased latency time (time taken by rats to enter to the dark chamber as compared to $AlCl_3$ rats). Cladodes extracts increased latency time better than peel and pulp extracts, and finally cladodes polysaccharides (Fig. 3).

3.6. Effect of extracts on brain monoamine neurotransmitter levels

The levels of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in brain homogenates were significantly reduced in the $AlCl_3$ group when compared to the untreated control group. Treatments of the rats with the extracts significantly restored the catecholamine levels (Table 4).

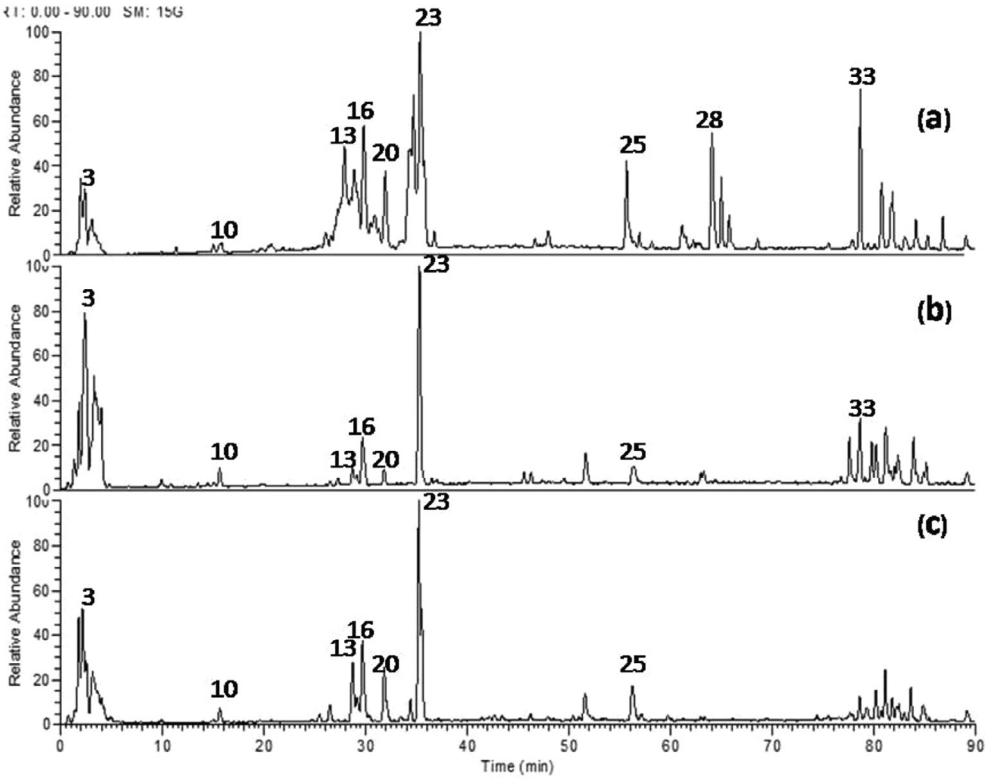

Fig. 1. HPLC-MS/MS profiles of *Opuntia ficus-indica* methanol extracts. (a) Cladode extract, (b) peel extract, (c) fruit pulp extract.

3.7. Effect of extracts on brain acetylcholinesterase (AChE) levels

Acetylcholinesterase is the key enzyme hydrolyzing the neurotransmitter acetylcholine. The level of acetylcholinesterase was significantly increased in $AlCl_3$ group when compared to the control group. All the studied extracts showed a significant decrease in AChE levels counteracting the effect of $AlCl_3$ (Table 5).

3.8. Effect of the extracts on neuro-inflammation markers in brain

Administration of $AlCl_3$ for six weeks strongly elevated the brain TNF-α and nuclear factor kappa B (NF-κβ) levels and decreased the interleukin-10 (IL-10) levels compared to the control group. All extracts decreased the brain TNF-α and NF-κβ and increased the reduced IL-10 levels (Fig. 4).

3.9. Molecular modeling

The scoring function and the interaction types between the docked compounds and the amino acid residues of the acetylcholinesterase (AChE) and serotonin transporter protein (SERT) are shown in Table 6.

In this study, some of the abundant compounds in the studied extracts, were docked into the crystal structures of acetylcholinesterase (AChE) and serotonin transporter protein (SERT), which function to regulate the neurotransmitters acetylcholine and serotonin respectively at the synapses of nervous system.

Progression of Alzheimer and other neurological disorders is accompanied by a reduction of acetylcholine in the brain leading to defects in cholinergic neurotransmission. Thus, inhibitors of AChE like the alkaloid galanthamine (from *Galanthus nivalis*) and the synthetic drug donepezil are used for symptomatic treatment of AD (Mehta et al., 2012; Muñoz-Torrero, 2008). The human AChE crystal structure in complex with donepezil has been recently resolved in which donepezil was shown to afford several polar and hydrophobic interactions with amino acid residues at the enzyme binding site. Hydrophobic interactions with the residues Trp 86, Trp 286, Phe 338, while the polar interactions (H-bond) involve the residues Asp 74, Tyr 124, Phe 295, Tyr 341, and the critical one to Tyr 337 (Cheung et al., 2012).

The selected compounds from the extracts were able to afford the majority of the previously reported interactions with an appreciable binding free energy reflected by a scoring function range of −32.39 to −17.69 kcal/mol (Table 6). In general, the glucoside derivatives exhibit better scoring functions and interactions with the amino acid residues at AChE binding site owing to their bulkier structure (Fig. S2 and Fig. 5). Two of the glucosides, namely, sinapic acid glucoside and 7-glucosyl-oxy-5-methylflavone glucoside were even able to interact with the critical Tyr 337 residue revealing strong AChE inhibitory potential (Fig. 5). Isorhamnetin, quercetin, and kaempferol aglycons have shown similar interaction fashion with the target enzyme and close scoring function values (Table 6).

Catecholamine and serotonin influence a diversity of neurological functions and processes in nervous system including cognition and memory functions (Berger et al., 2009). The reuptake of these neurotransmitters leads to signaling termination and is mediated by a family of sodium symporter transporters including serotonin transporter (SERT), dopamine transporter (DAT), and norepinephrine transporter (NET). Drugs interfering with these transporters elevate biogenic amines brain level in the synaptic cleft and thus used to alleviate many psychiatric disorders (Berger et al., 2009; Bröer and Gether, 2012).

Recently, the human SERT crystallographic X-ray structure in complex with the antidepressants paroxetine and escitalopram has been reported and revealed central and allosteric binding sites (Coleman et al., 2016). Paroxetine binds only to the central site and affords different hydrogen bond and hydrophobic interactions with several amino acids, most importantly, Asp 98, Tyr 95, Tyr 176, Ile

Table 1
Chemical composition of *Opuntia ficus-indica* extracts.

No.	Tentatively identified compounds	Rt (min)	$[M-H]^-$	MS/MS	Extract			Ref.
					Cladodes	Fruit peel	Fruit pulp	
1	Quinic acid[a]	1.38	191		0.10	5.87	0.84	
2	Malic acid[a]	1.66	133	115	3.02	1.99	0.01	
3	Piscidic acid[a,b]	1.95	255	193, 165	7.02	10.40	9.67	(Petruk et al., 2017)
4	Diferuloyl-syringsic acid[b]	2.17	549	255	23.37	10.40	9.67	(Benayad et al., 2014)
5	Eucomic acid[a,b]	2.78	239	149, 179, 221	11.62	10.40	16.25	(Petruk et al., 2017)
6	Dicaffeoylferulic acid[b]	3.02	517	239	5.81	17.48	16.25	(Benayad et al., 2014)
7	*p*-Coumaric acid 3-*O*-glucoside[a]	4.06	325	163	0.44	16.34	Nd	
8	7-Glucosyl-oxy-5-methyl flavone glucoside	14.70	611	251, 431	0.04	0.45	Nd	
9	Sinapic acid 3-*O*-glucoside[a]	14.91	385	223	0.40	Nq	8.74	(Sobeh et al., 2017a)
10	Sinapic acid 3-*O*-galactoside[a]	15.63	385	223	0.31	Nq	Nd	
11	Quercetin pentosyl-rutinoside[b]	26.02	741	271, 301, 609	0.32	0.15	0.64	
12	Kaempferol rhamnosyl-rutinoside	26.16	739	255, 285, 593	0.32	0.84		
13	Isorhamnetin glucosyl-rutinoside[b]	26.7	785	300, 315, 623	1.9	0.04	1.79	(Benayad et al., 2014)
14	Rhamnetin rhamnosyl-rutinoside[b]	27.87	769	315, 477, 623	7.86	1.40	Nd	(Benayad et al., 2014)
15	Isorhamnetin rhamnosyl-rutinoside[b]	29.08	769	315, 477, 623	7.86	2.1	Nd	(Benayad et al., 2014)
16	Isorhamnetin pentosyl-rutinoside[b]	29.20	755	315, 461, 623	5.54	7.37	8.85	(Benayad et al.. 2014)
17	Quercetin rutinoside (rutin) [a]	29.36	609	179, 271, 301	20.24	0.98	Nd	
18	Kaempferol pentosyl-rutinoside[b]	29.50	725	255, 285, 593	25.26	Nq	Nd	(Benayad et al., 2014)
19	Isorhamnetin pentosyl-rutinoside	30.00	755	315, 461, 623	3.5	Nq	10	
20	Isorhamnetin pentosyl-hexoside[b]	31.91	609	179, 300, 315	2.36	1.97	1.48	(Benayad et al., 2014)
21	Isorhamnetin rutinoside[a,b]	34.68	623	179, 300, 315	16.7	29.7	Nd	(Benayad et al., 2014)
22	Rhamnetin 3-*O*-glucoside[a,b]	35.71	477	151, 179, 315	2.88	0.84	1.67	(Benayad et al., 2014)
23	Isorhamnetin 3-*O*-glucoside[a,b]	36.78	477	151, 179, 315	2	0.84	Nd	(Benayad et al., 2014)
24	Isorhamnetin coumaroyl-rutinoside[b]	47.85	785	271, 314, 623	4.37	0.05	Nd	(Benayad et al., 2014)
25	Rhamnetin[a]	55.69	315	179, 300	1.6	Nq	Nd	
26	Isorhamnetin[a]	61.15	315	179, 300, 315	9.2	3.3	2.9	
27	Diosmetin[a]	63.96	299	179, 271, 284	5.9	Nq	Nd	
28	Tricin[a]	65.00	329	314, 329	21.3	4.23	Nd	
29	Unknown	65.68	555	225, 299, 555	13.6	6.57	7.14	
30	Unknown	78.69	513	253, 277, 513	4.7	Nq	0.01	
31	Hydroxyl octadecadienoic acid	79.4	295	171, 295	1.81	Nq	Nd	
32	Unknown	80.03	515	253	1.72	Nq	Nd	
33	Eicosanoic acid[a]	81.68	311	311	8.5	12.20	Nd	
34	Eicosanoic acid isomer	82.95	311	311	9	15.19	Nd	
35	Heneicosanoic acid	84.05	325	325	12.49	6.94	7.65	
36	Eicosanoic acid isomer	85.31	311	311	8.5	4.23	Nd	
37	Behenic acid[a]	86.76	339	339	10.59	6.55	Nd	

Nq: not quantified, Nd: not detected.
[a] Compounds were confirmed using authentic compounds.
[b] Compounds were previously described from the plant.

Table 2
Polysaccharides hydrolysate of *Opuntia ficus-indica* mucilage from fruit and cladodes pulp.

Identified sugar	Percentage in mucilage	
	Fruit pulp	Cladodes pulp
Glucuronic acid	77.79	73.11
Galacturonic acid	5.35	5.63
Glucose	0.036	0.063
Galactose	0.097	0.12
Rhamnose	0.19	0.13
Fructose	0.33	0.31
Unidentified	16.21	20.63

Table 3
Antioxidant potential of OFI extracts compared to the positive control vitamin C.

Extract	DPPH [IC_{50}, μg/mL]
Cladode extract	6.70 ± 0.034
Fruit peel extract	10.65 ± 0.054
Fruit pulp extract	16.57 ± 0.078
Polysaccharides	74.13 ± 0.092
Vitamin C	3.15 ± 0.082

172, Phe 341, Phe 335, and Thr 497. Escitalopram affords similar interactions with the central site and binds also to the allosteric site through interacting with the amino acids Asp 328, Arg 104, Gln 332, Ala 331, and Phe 556 (Coleman et al., 2016).

As shown in Table 6, the selected compounds from the extracts have been able to bind to SERT binding sites with a scoring function range of −17.84 to −12.03 kcal/mol. The free aglycones along with isorhamnetin glucoside have bound to the central binding site affording similar interactions reported by paroxetine. The sinapic acid glucoside has interacted with amino acid residues at the allosteric site only, while 7-glucosyl-oxy-5-methylflavone glucoside has fitted mainly in the allosteric site and extended to afford some interactions with residues in the central binding site (Fig. S2).

Because these compounds are partly ionized at the physiological pH we have considered docking the ionized form (phenolate ion) to AChE and SERT binding sites which revealed ionic interaction with basic amino acids such as arginine and lysine (Fig. S3).

4. Discussion

The phytochemical constituents of different parts from *Opuntia ficus-indica* were characterized using HPLC-MS/MS and their neuroprotective activity against $AlCl_3$ induced neurotoxicity in rats was evaluated. A total of 37 secondary metabolites, mainly isorhamnetin glycosides, were identified. A daily oral dose of 100 mg/kg of the extracts for six weeks was able to counteract the $AlCl_3$ induced neuro-inflammation and oxidative stress. They restore the brain monoamine neurotransmitter and acetylcholinesterase levels and enhance learning and memory functions of the experimental animals.

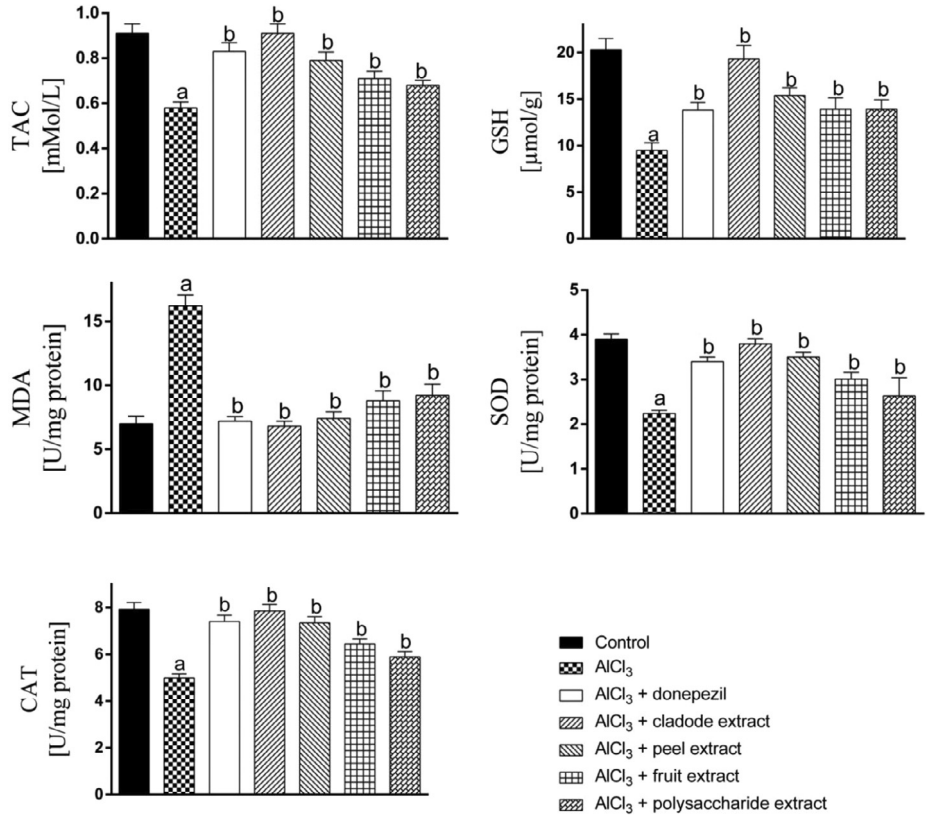

Fig. 2. Effect of OFI extracts on oxidative stress markers in $AlCl_3$ rats. (a) Total antioxidant capacity (TAC), (b) reduced glutathione (GSH), (c) Malondialdhyde (MDA), (d) superoxide dismutase (SOD), (e) catalase (CAT). Values are expressed as mean ± SE for 8 rats in each group, [a]$p < 0.05$ versus control and [b]$p < 0.05$ versus $AlCl_3$ group.

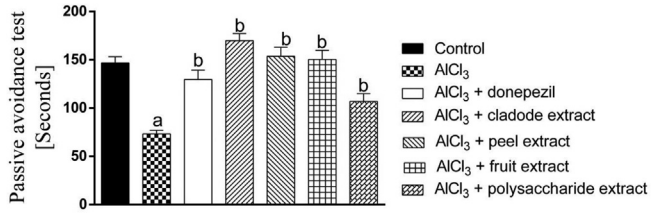

Fig. 3. Effect of OFI extracts on latency time in $AlCl_3$ rats. Cladode and fruit peel extracts exhibited similar activities like the drug donepezil. Data are shown as mean ± SE for 8 rats in each group. [a]$p < 0.05$ versus control and [b]$p < 0.05$ versus $AlCl_3$ group.

Table 4
Effects of OFI extracts on brain neurotransmitter levels in $AlCl_3$ rats: serotonin, dopamine, norepinephrine. Cladodes extract showed approximately similar activities like the reference drug donepezil.

Groups	Noradrenalin	Dopamine	Serotonin
	(µg/g brain)		
Control	0.62 ± 0.06	1.68 ± 0.05	0.54 ± 0.03
$AlCl_3$	0.33 ± 0.02[a]	1.0 ± 0.04[a]	0.25 ± 0.02[a]
$AlCl_3$ + Donepezil	0.59 ± 0.03[b]	1.6 ± 0.1[b]	0.46 ± 0.03[b]
$AlCl_3$ + Cladode extract	0.57 ± 0.03[b]	1.53 ± 0.05[b]	0.44 ± 0.03[b]
$AlCl_3$ + Peel extract	0.48 ± 0.03[b]	1.37 ± 0.06[b]	0.41 ± 0.03[b]
$AlCl_3$ + Fruits extract	0.44 ± 0.03[b]	1.25 ± 0.06[b]	0.38 ± 0.03[b]
$AlCl_3$ + Polysaccharides	0.40 ± 0.03[b]	1.11 ± 0.07[b]	0.33 ± 0.03[b]

Values are shown as mean ± SE for 8 rats in each group.
[a] $p < 0.05$ versus control.
[b] $p < 0.05$ versus $AlCl_3$ group.

Table 5
Effect of OFI extracts on acetylcholinesterase (AChE) levels in $AlCl_3$ rats.

Groups	AChE (ng/g brain)
Control	0.82 ± 0.07
$AlCl_3$	4.48 ± 0.44[a]
$AlCl_3$ + Donepezil	0.79 ± 0.05[b]
$AlCl_3$ + Cladode extract	0.90 ± 0.05[b]
$AlCl_3$ + Peel extract	1.98 ± 0.16[a,b]
$AlCl_3$ + Fruits extract	3.05 ± 0.22[a,b]
$AlCl_3$ + Polysaccharides	4.14 ± 0.32[a]

Data are shown as mean ± SE for 8 rats in each group.
[a] $p < 0.05$ versus control.
[b] $p < 0.05$ versus $AlCl_3$ group.

The potential of flavonoids (as glucosides or free aglycones) and polyphenols to transverse the blood brain barrier has been widely investigated. Compounds like catechin, quercetin, naringenin, chrysanthemin (cyaniding-3-glucoside), and others were reported to be capable of crossing blood brain barrier in cell models and *in vivo* experiments (Youdim et al., 2003; Faria et al., 2010). The possible mechanisms include passive diffusion and active transport - by virtue of the glucose transporters - which is mainly restricted to glucosides owing to the glucose moiety in their structure (Faria et al., 2009).

$AlCl_3$ can induce biochemical and neurochemical abnormalities including oxidative brain injury, induction of apoptosis and neuronal damage, neuralgia inflammatory reaction, and reduced neurotransmitter biosynthesis (Singla and Dhawan, 2014, Kim et al., 2018). The administered dose of the extracts resulted in significant increase in latency time compared to $AlCl_3$ rats.

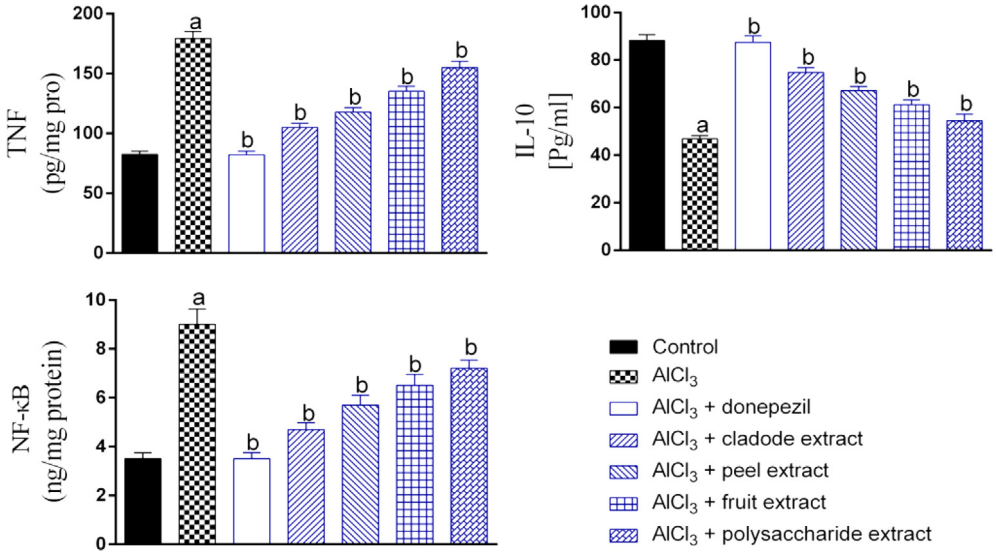

Fig. 4. Effects of OFI extracts on brain neuro-inflammation marker levels in $AlCl_3$ rats. (a) Tumor necrosis factor (TNFα), (b) nuclear factor kappa B (NF-κβ) level, (c) interleukin-10 (IL-10) level. Values are represented as mean ± SE for 8 rats in each group. $^{a}p < 0.05$ versus control and $^{b}p < 0.05$ versus $AlCl_3$ group.

Table 6
Docking scoring functions (kcal/mol) and compound-amino acid residues interactions in acetylcholinesterase and serotonin transporter proteins.

Compound	Acetylcholinesterase		Serotonin Transporter Protein	
	Scoring function (kcal/mol)	Amino acids interactions	Scoring function (kcal/mol)	Amino acids interactions
Sinapic acid glucoside	−20.68	Trp 286: H-bond Tyr 337: H-bond Tyr 341: H-bond Trp 86: Hydrophobic Trp 286: Hydrophobic	− 13.02	Arg 104: H-bond Gln 332: H-bond Lys 490: H-bond Glu 494: Hydrophobic
Isorhamnetin	−18.31	Ser 293: H-bond Arg 296: H-bond Tyr 341: Hydrophobic	− 12.39	Phe 335: H-bond Ile 172: Hydrophobic Tyr 176: Hydrophobic Gly442: Hydrophobic
Isorhamnetin glucoside	−27.49	Tyr 72: H-bond Asp 74: H-bond Phe 297: H-bond Trp 86: Hydrophobic Trp 286: Hydrophobic	− 17.84	Tyr 176:H-bond Phe 335: H-bond Thr 497: H-bond Tyr 95: Hydrophobic Tyr 176: Hydrophobic
Quercetin	−19.75	Asp 74: H-bond Thr 83: H-bond Phe 338: Hydrophobic Tyr 341: Hydrophobic	− 13.66	Tyr 176: Hydrophobic Phe 341: Hydrophobic Gly442: Hydrophobic
Kaempferol	−17.69	Tyr 72: H-bond Asp 74: H-bond Tyr 341: H-bond Ser 293: H-bond Arg 296: H-bond	− 12.03	Ile 172: Hydrophobic Tyr 176: Hydrophobic Gly 442: Hydrophobic
7-glucosyl-oxy-5-methylflavone glucoside	−32.39	Tyr 341: H-bond Ser 293: H-bond Phe 295: H-bond Trp 86: Hydrophobic Tyr 337: Hydrophobic Tyr 341: Hydrophobic	− 14.22	Asp 98: H-bond Arg 104: H-bond Thr 497: H-bond Phe 556: Hydrophobic

Monoamine neurotransmitters, namely NE, DA and 5-HT are considered to be closely involved in memory and learning. Deficiency in the level of these neurotransmitters in the hippocampus leads to the cerebral impairment and plays a role in early pathological process of AD (Singh et al., 2013). The levels of monoamine neurotransmitters were significantly reduced in $AlCl_3$ rats as compared to the untreated control group. Significant restoration in the levels of NE, DA and 5-HT was observed in rats treated with the extracts revealing an improved memory function. Acetylcholine is a crucial neurotransmitter involved in regulating cognitive function. Therefore, the inhibition of AChE is considered a substantial strategy for treating neurological disorders. The level of AChE was significantly elevated in $AlCl_3$ rats as compared to the untreated control group. Significant reduction in the level of AChE was observed in rats treated with the extracts revealing promising neuroprotective activities.

Oxidative stress is a major factor contributing to the initiation and progress of several neurological disorders, among them AD (Dumont and Beal, 2011). All OFI extracts were able to reduce the $AlCl_3$ induced oxidative stress in the brain where they demonstrated a significant decrease in oxidative stress marker MDA level and significant increase in TAC, GSH, SOD and CAT levels revealing

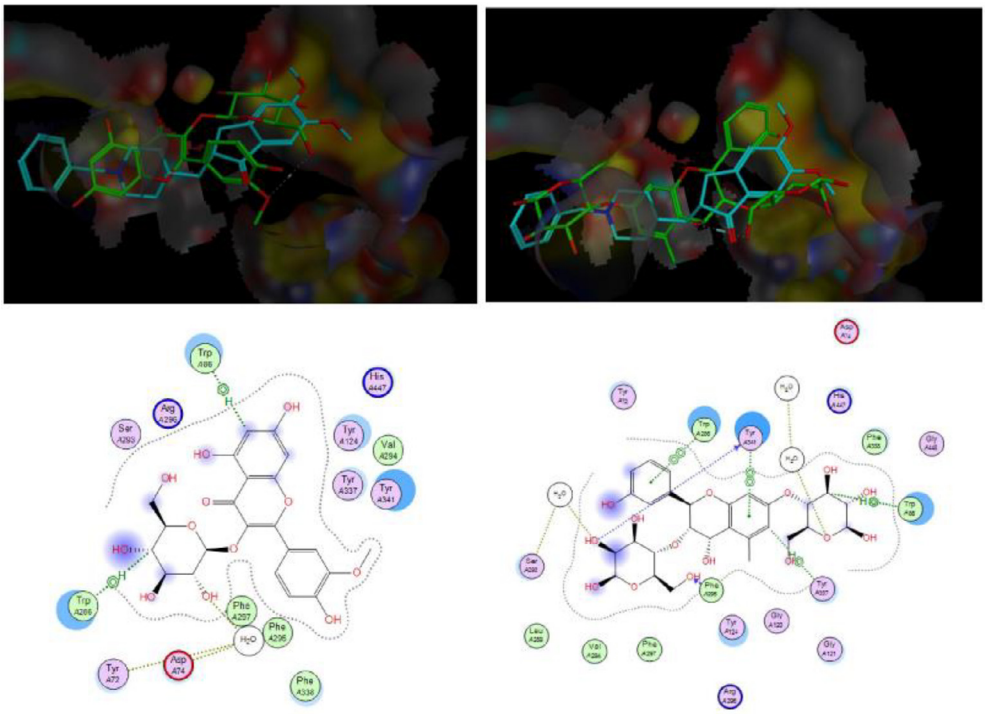

Fig. 5. Docking of isorhamnetin glucoside (3D: top left, 2D: bottom left) and 7-glucosyl-oxy-5-methylflavone glucoside (3D: top right, 2D: bottom right) to AChE binding site. The docked compounds are labeled green, while the co-crystallized reference drug donepezil is cyan blue.

substantial antioxidant activities *in vivo*. Similar activities were reported from OFI fruits and were attributed to the antioxidant action of the extract (Ha et al., 2003).

Beside oxidative stress, neuro-inflammation is an early event in AD. All the studied extracts demonstrated significant decrease in brain NF-κβ and TNF-α levels and significant increase in IL-10 levels. Our results suggest that OFI decrease the $AlCl_3$ induced neuro-inflammation in the brain. Similar properties were shown by (Kim et al., 2010; Kim et al., 2006) where OFI extracts attenuated neuronal injury in rats of cerebral ischemia.

Molecular modeling study provided an insight about the molecular targets, which could be plausibly involved in the mechanism of action of the studied OFI extracts. Docking results suggested that the extracts' components could serve as AChE and SERT inhibitors which come in agreement with a previous study that has determined the AChE inhibitory activity of some OFI extracts obtained by various extraction methods and reported IC_{50} value of 0.78 mg/ml for the ethanol extract (Ressaissi et al., 2016). In the same context, certain quercetin and isorhamnetin glucosides have also reported to exhibit appreciable activity (Olennikov et al., 2017). Moreover, several studies have reported the inhibitory effect of various polyphenols of different sources like green tea and licorice on the monoamine transporter proteins (Olennikov et al., 2017; Ofir et al., 2003; Yáñez et al., 2006).

5. Conclusions

Utilizing LC-MS/MS, 37 secondary metabolites were tentatively identified in *Opuntia ficus-indica* extracts. In DPPH assays, the extracts exhibited substantial antioxidant activities. *In vivo*, the extracts demonstrated substantial neuroprotective activity against $AlCl_3$ induced neurotoxicity. In conclusion, *Opuntia ficus-indica* counteracts oxidative stress and might be an interesting candidate for the treatment of several neurological disorders, which needs to be studied in more detail in other experimental systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We acknowledge the financial support by UM6P for funding Open Access Publishing.

References

Ahmed, F., Omar, A.H., Ahmed, Rania F., Saleh, Dalia O., 2016. Combination of resveratrol and fluoxetine in an acute model of depression in mice: prevention of oxidative DNA fragmentation and monoamines degradation. J. Appl. Pharmaceut. Sci. 1, 001–007.

Ahmed, M., El Tanbouly, N., Islam, W., Sleem, A., El Senousy, A., 2005. Antiinflammatory flavonoids from *Opuntia dillenii* (Ker-Gawl) Haw. flowers growing in Egypt. Phytother. Res. 19, 807–809.

Ali, A.A., Ahmed, H.I., Zaky, H.S., 2016. Stress as a risk factor in induction and progression of alzheimer's disease: Impact on the possible protection using epigallocatechin-3-gallate and/or diazepam. Alzheimer's Dement 12, 1027–1028.

Ammar, I., Ennouri, M., Attia, H., 2015. Phenolic content and antioxidant activity of cactus (*Opuntia ficus-indica* L.) flowers are modified according to the extraction method. Ind. Crops Prod. 64, 97–104.

Barba, F.J., Putnik, P., Bursać Kovačević, D., Poojary, M.M., Roohinejad, S., Lorenzo, J. M., Koubaa, M., 2017. Impact of conventional and non-conventional processing on prickly pear (*Opuntia* spp.) and their derived products: From preservation of beverages to valorization of by-products. Trends Food Sci. Technol. 67, 260–270.

Benayad, Z., Martinez-Villaluenga, C., Frias, J., Gomez-Cordoves, C., Es-Safi, N.E., 2014. Phenolic composition, antioxidant and anti-inflammatory activities of extracts from Moroccan *Opuntia ficus-indica* flowers obtained by different extraction methods. Ind. Crops Prod. 62, 412–420.

Berger, M., Gray, J.A., Roth, B.L., 2009. The expanded biology of serotonin. Annu. Rev. Med. 60, 355–366.

Bröer, S., Gether, U., 2012. The solute carrier 6 family of transporters. Br. J. Pharmacol. 167, 256–278.

Chaouch, M.A., Hafsa, J., Rihouey, C., Le Cerf, D., Majdoub, H., 2016a. Effect of extraction conditions on the antioxidant and antiglycation capacity of

carbohydrates from Opuntia robusta cladodes. Int. J. Food Sci. Technol. 51, 929–937.
Chaouch, M.A., Hafsa, J., Rihouey, C., Le Cerf, D., Majdoub, H., 2016b. Effect of pH during extraction on the antioxidant and antiglycated activities of polysaccharides from opuntia ficus indica. J. Food Biochem. 40, 316–325.
Cheung, J., Rudolph, M.J., Burshteyn, F., Cassidy, M.S., Gary, E.N., Love, J., Franklin, M. C., Height, J.J., 2012. Structures of human acetylcholinesterase in complex with pharmacologically important ligands. J. Med. Chem. 55, 10282–10286.
Coleman, J.A., Green, E.M., Gouaux, E., 2016. X-ray structures and mechanism of the human serotonin transporter. Nature 532, 334–339.
Dumont, M., Beal, M.F., 2011. Neuroprotective strategies involving ROS in Alzheimer disease. Free Radical Biol. Med. 51, 1014–1026.
El Hawary, S.S., Saad, S., El Halawany, A.M., Ali, Z.Y., El Bishbishy, M., 2016. Phenolic content and anti-hyperglycemic activity of pecan cultivars from Egypt. Pharm. Biol. 54, 788–798.
El-Hawary, S.S., Ali, Z.Y., Younis, I.Y., 2018. Hepatoprotective potential of standardized *Ficus* species in intrahepatic cholestasis rat model: Involvement of nuclear factor-κB, and farnesoid X receptor signaling pathways. J. Ethnopharmacol. https://doi.org/10.1016/J.JEP.2018.11.026.
El Tanbouly, N., El Sayed, A.M., Ali, Z.Y., Abdel Wahab, S., El Gayed, S.H., Ezzat, S.M., El Senousy, A.S., Choucry, M.A., Abdel-Sattar, E., 2017. Antidepressant-like effect of selected Egyptian cultivars of flaxseed oil on a rodent model of postpartum depression. Evidence Based Complement. Alternative Med. 2017. https://doi.org/10.1155/2017/6405789.
European Pharmacopeia, 2002. 4th Ed. Council of Europe Press, Strasburg.
Faria, A., Pestana, D., Azevedo, J., Martel, F., de Freitas, V., Azevedo, I., Mateus, N., Calhau, C., 2009. Absorption of anthocyanins through intestinal epithelial cells–Putative involvement of GLUT2. Mol. Nutr. Food Res. 53, 1430–1437.
Faria, A., Pestana, D., Teixeira, D., Azevedo, J., Freitas, V., Mateus, N., Calhau, C., 2010. Flavonoid transport across RBE4 cells: a blood-brain barrier model. Cell. Mol. Biol. Lett. 15, 234–241.
Feldman, R.S., Quenzer, L.F., 1984. Fundamentals of Neuropsychopharmacology. Sinauer Associates Sunderland, MA.
Galati, E.M., Mondello, M.R., Giuffrida, D., Dugo, G., Miceli, N., Pergolizzi, S., Taviano, M.F., 2003. Chemical characterization and biological effects of Sicilian *Opuntia ficus-indica* (L.) Mill. fruit juice: antioxidant and antiulcerogenic activity. J. Agric. Food. Chem. 51, 4903–4908.
Ghareeb, M.A., Mohamed, T., Saad, A.M., Refahy, L.A.-G., Sobeh, M., Wink, M., 2018. HPLC-DAD-ESI-MS/MS analysis of fruits from *Firmiana simplex* (L.) and evaluation of their antioxidant and antigenotoxic properties. J. Pharm. Pharmacol. 70, 133–142.
Ha, H.J., Kwon, Y.S., Park, S.M., Shin, T., Park, J.H., Kim, H.C., Kwon, M.S., Wie, M.B., 2003. Quercetin attenuates oxygen–glucose deprivation-and excitotoxin-induced neurotoxicity in primary cortical cell cultures. Biol. Pharm. Bull. 26, 544–546.
Kim, J.-H., Park, S.-M., Ha, H.-J., Moon, C.-J., Shin, T.-K., Kim, J.-M., Lee, N.-H., Kim, H.-C., Jang, K.-J., Wie, M.-B., 2006. *Opuntia ficus-indica* attenuates neuronal injury in in vitro and in vivo models of cerebral ischemia. J. Ethnopharmacol. 104, 257–262.
Kim, J.M., Kim, D.H., Park, S.J., Park, D.H., Jung, S.Y., Kim, H.J., Lee, Y.S., Jin, C., Ryu, J. H., 2010. The n-butanolic extract of *Opuntia ficus-indica* var. saboten enhances long-term memory in the passive avoidance task in mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 34, 1011–1017.
Kim, H.J., Jung, S.W., Kim, S.Y., Cho, I.H., Kim, H.C., Rhim, H., Kim, M., Nah, S.Y., 2018. Panax ginseng as an adjuvant treatment for Alzheimer's disease. J. Ginseng Res., 1–11 https://doi.org/10.1016/j.jgr.2017.12.008.
Kumazawa, S., Tanguchi, M., Suzuki, Y., Shimura, M., Kwon, M.S., Nakayama, T., 2002. Antioxidant activity of polyphenols in carob pods". J. Agric. Food. Chem. 50 (2), 373–377.
Laidlaw, R., Percival, E., 1950. 101. Studies of seed mucilage. Part V. Examination of a polysaccharide extracted from the seeds of *Planta goovata* Forsk by hot water. J. Chem. Soc., 528–534
Mehta, M., Adem, A., Sabbagh, M., 2012. New acetylcholinesterase inhibitors for Alzheimer's disease. Int. J. Alzheimer's Dis.
Muñoz-Torrero, D., 2008. Acetylcholinesterase inhibitors as disease-modifying therapies for Alzheimer's disease. Curr. Med. Chem. 15, 2433–2455.
OECD, 2001. OECD/OCDE 423. OECD guideline for testing of chemicals acute oral toxicity.
Ofir, R., Tamir, S., Khatib, S., Vaya, J., 2003. Inhibition of serotonin re-uptake by licorice constituents. J. Mol. Neurosci. 20, 135–140.
Olennikov, D.N., Kashchenko, N.I., Chirikova, N.K., Akobirshoeva, A., Zilfikarov, I.N., Vennos, C., 2017. Isorhamnetin and quercetin derivatives as anti-acetylcholinesterase principles of marigold (*Calendula officinalis*) flowers and preparations. Int. J. Mol. Sci. https://doi.org/10.3390/ijms18081685.
Pazur, J., Chaplin, N., Kennedy, J., 1986. Carbohydrate Analysis: A Practical Approach. Oxford Press, London.
Petruk, G., Di Lorenzo, F., Imbimbo, P., Silipo, A., Bonina, A., Rizza, L., Piccoli, R., Monti, D.M., Lanzetta, R., 2017. Protective effect of *Opuntia ficus-indica* L. cladodes against UVA-induced oxidative stress in normal human keratinocytes. Bioorg. Med. Chem. Lett. 27, 5485–5489.
Prema, A., Thenmozhi, A.J., Manivasagam, T., Essa, M.M., Akbar, M.D., Akbar, M., 2016. Fenugreek seed powder nullified aluminium chloride induced memory loss, biochemical changes, a β burden and apoptosis via regulating Akt / GSK3 β Signaling. PLOS one 1, 1–19.
Ressaissi, A., Attia, N., Falé, P., Pacheco, R., Teixeira, V., Machuqueiro, M., Borges, C., Serralheiro, M., 2016. Aqueous extracts from nopal (*Opuntia ficus-indica*): anti acetylcholinesterase and antioxidant activity from phenolic bioactive compounds. Int. J. Green Herbal Chem. 5, 337–348.
Shin, C.Y., Kim, H.S., Cha, K.H., Won, D.H., Lee, J.Y., Jang, S.W., Sohn, U.D., 2018. The effects of Donepezil, an acetylcholinesterase inhibitor, on impaired learning and memory in rodents. Biomol. Therap. 26, 274.
Singh, C., Bortolato, M., Bali, N., Godar, S.C., Scott, A.L., Chen, K., Thompson, R.F., Shih, J.C., 2013. Cognitive abnormalities and hippocampal alterations in monoamine oxidase A and B knockout mice. Proc. Natl. Acad. Sci. 110, 12816–12821.
Singla, N., Dhawan, D.K., 2014. Influence of zinc on calcium-dependent signal transduction pathways during aluminium-induced neurodegeneration. Mol. Neurobiol. 50, 613–625. https://doi.org/10.1007/s12035-014-8643-7.
Sobeh, M., Hassan, S., El Raey, M., Khalil, W., Hassan, M., Wink, M., 2017a. Polyphenolics from *Albizia harveyi* exhibit antioxidant activities and counteract oxidative damage and ultra-structural changes of cryopreserved bull semen. Molecules 22, 1993.
Sobeh, M., Mahmoud, M.F., Abdelfattah, M.A.O., El-Beshbishy, H.A., El-Shazly, A.M., Wink, M., 2017b. Hepatoprotective and hypoglycemic effects of a tannin rich extract from *Ximenia americana* var. *caffra* root. Phytomedicine 33, 36–42.
Stintzing, F.C., Herbach, K.M., Mosshammer, M.R., Carle, R., Yi, W., Sellappan, S., Akoh, C.C., Bunch, R., Felker, P., Color, betalain pattern, and antioxidant properties of cactus pear (Opuntia spp.) clones. J. Agric. Food Chem. 53, 442–451.
Stintzing, F., Carle, R., 2006. Cactus fruits–more than colour. Fruit Proc. 16, 166–171.
van Wyk, B.-E., Wink, M., 2015. Phytomedicines, Herbal Drugs, and Poisons. University of Chicago Press.
Van Wyk, B.-E. and Wink, M., Medicinal Plants of the World, 2017, Briza, Pretoria.
White, E., Rao, P., 1953. Constitution of the polysaccharide from tamarind seed. J. Am. Chem. Soc. 75, 2617–2619.
Yáñez, M., Fraiz, N., Cano, E., Orallo, F., 2006. Inhibitory effects of cis-and trans-resveratrol on noradrenaline and 5-hydroxytryptamine uptake and on monoamine oxidase activity. Biochem. Biophys. Res. Commun. 344, 688–695.
Youdim, K.A., Dobbie, M.S., Kuhnle, G., Proteggente, A.R., Abbott, N.J., Rice-Evans, C., 2003. Interaction between flavonoids and the blood–brain barrier: in vitro studies. J. Neurochem. 85, 180–192.

Further Reading

Gawryluk, J.W., Wang, J., Andreazza, A.C., Shao, L., Young, L.T., 2011. Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. Int. J. Neuropsychopharmacol. 14, 123–130.