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Ameliorative Role of Nutraceutical Quercetin and its Derivatives Against Cognitive Impairment Process Induced by Lead Exposure in *Drosophila melanogaster*(Fruit Fly)

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Abstract

Cumulative lifetime lead (Pb) exposure has been associated with accelerated declines in cognition through free radical generation and epigenetic effects.Several literature has established the link between lead exposure and neurodegenerative disorders.Harwich strain of drosophila Melanogaster were exposed to lead acetate for two weeks and the changes in impulse transmission through acetylcholinesterase and systemic redox state were assessed. In addition, molecular docking studies of acetylcholinesterase against quercetin was carried out. In silico toxicity, pharmacokinetics studies on quercetin were also carried-out. The data obtained showed alteration in function of antioxidant enzymes and molecules such as catalase, glutathione-S-transferase and glutathione. Up regulation of acetylcholinesterase activity was observed following treatment with quercetin. Molecular docking studies revealed quercetin to bind to both active and peripheral pocket of acetylcholinesterase. Pharmacokinetic studies show moderate solubility, high therapeutic index, excellent absorption capacity, hepatoprotective and non-mutagenic properties. Therefore, quercetin alongside other antioxidant molecules can play a vital role in preventing the onset of Alzheimer and antioxidant related disorders.

Keywords: Alzheimer; Antioxidant; Acetylcholinesterase; Neurodegeneration; Oxidative damage.

Introduction

Neurodegenerative disorders are age-related neurological disorders associated with the progressive loss of neurons' structure and function [(1,2)]. Commonly known among these disorders include Alzheimer's disease, Parkinson's disease, the foam of dementia, and Huntington's disease[(3)]. Alzheimer's disease is the most predominant among the elderly and fully characterized by extracellular plaques of β-amyloid, hyperphosphorylated tau protein, and loss of cholinergic neurons leading to a behavioral loss and cognitive function [(4)]. Several hypotheses have been put forward to explain this disorder, but the cholinergic hypothesis, which described the involvement of acetylcholinesterase and butyrylcholinesterase and oxidative stress hypothesis, were more prominent [(3,5)]. Evidence has proven the neuroprotective role of acetylcholinesterase inhibitors in the clinical management of Alzheimer's diseases. Some of these inhibitors reported include donepezil, galantamine, tacrine, neostigmine, pyridostigmine, and physostigmine, among many other compounds and plant extracts [(6,7)]. The role of oxidative damage in the pathophysiology of Alzheimer's disease and other neurodegenerative disorders has been fully documented [(8)]. Quercetin is a natural flavonoid found abundantly in almost all edible vegetables and fruits such as red onion, common onion, cranberry, blueberry[Figure 1] [(8)]. Intake of a quercetin-rich diet is highly encourage and is positively correlated with health improvement[(10)]. It can also be taken as dietary supplement with daily recommended doses of 200–1200 mg as well as a nutraceutical through functional foods with a concentration range of 10–125 mg per serving [(11)]. Several studies suggest quercetin therapeutic potential and its derivatives to prevent and treat various chronic diseases, including cardiovascular, neurodegenerative, and cancer [(12-14)].

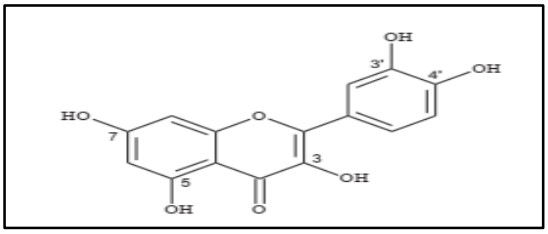

Figure 1. Structure of Quercetin

Lead (Pb) is one of the most abundant heavy metal pollutants in the environment and it's considered to be one of the most hazardous chemicals for humans and animals health [(15)].

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Lead exposure has been attributed to several clinical symptoms including arthritis, renal dysfunction, birth defects, mental retardation, psychosis, hyperactivity, autism and brain damage [16]. And, several neurodegenerative disorders such as Huntingtons syndrome, Parkinson and Alzheimer diseases models were simulated in drosophila [17-19], the organism has contributed significantly to the understanding of processes affecting metal toxicity [20]. It is therefore considered to be a useful model species for the investigation of biological reactions to toxic chemicals [21,22,15], with its genes having many structurally and functionally preserved homologies to humans [23]. This study aimed to determine the effect of short-term exposure to acetylcholinesterase activity in relation to oxidative damage associated with Alzheimer's disease pathogenesis and the role of functional foods in improving these oxidative processes.

Materials and Methods

The Randox Protein Kit was Purchased from Medicom, State of Jos Plateau. Sigma Aldrich purchased 1-chloro-2, 4-dinitrobenzene (CDNB), and 5-dithiobis (2-nitrobenzoic acid)(DTNB) (St Louis, MO). Harwich strain flies were collected from Jos, Plateau State, African Center of Excellence for Phytomedicine Research and Development.

Grouping and Treatments

The flies were cultured on the standard Drosophila culture medium consisting of corn flour, brown sugar, yeast, agar, and propionic acid as a mould inhibitor. The culture was maintained at 25±2°C in controlled 12 hour light and darkness. Lead acetate (300 µL) was used as model pollutant and the flies were divided into four groups. Group one was treated with normal feed containing normal distilled water, group two with quercetin (100 µL), group three lead acetate (300 µL) and group four with the combination of lead acetate and quercetin in stated concentration. The flies were anesthetized to ice and homogenized to 100 mM phosphate buffer saline (pH7.4) in 1:10 volumes. The flies were centrifugated with a cold centrifuge at 4000 rpm.

Determination of total Thiol content

The total thiol content was calculated using Ellman's process [24]. The reaction mix comprised 510 µL potassium phosphate buffer (0.1 M, PH 7.4), 25 µL sample, and 30 µL DTNB (10 mM). The reaction mixture was incubated for 30 min at room temperature, the optical density was taken at 412 nm and total thiol content was expressed in mmol/mg protein. Standard glutathione was used for standard calibration. According to the manufacturers' instructions, the protein concentration of homogenates was determined with the total protein kit (Randox). The data were measured with blank and blank samples, and the results were all corrected with protein content.

Determination of Glutathione-S-transferase (GST) activity

Activity of Glutathione-S-transferase (GST; EC 2.5.1.18) was calculated using Habig and Jacoby method [25], with 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. The reaction mix consist solution A 600 µL, (20 µL 0.25 M potassium phosphate buffer, pH 7.0 with 2.5 mM EDTA and 510 µL 0.1 M GSH at 25°C), 60 µL sample (1:5 dilution) and 30 µL 25 mM CDNB. Optical density was calculated at 340 nm at 10 s intervals for 2 min (Jenway). The data were expressed in mmol/min using the 9.6 mM1 cm1 molar extinction coefficient of the GST-formed GS–DNB conjugate.

Determination of Catalase (CAT) Activity

The activity of catalase (CAT; EC 1.11.1.6) was monitored using a process of Aebi [26]. Reaction mix containing 100 mL of potassium phosphate buffer (pH 7.0), 194 mL of solution A (300 mM H_2O_2). About 10 µL of sample was added to 590 µL of solution A and the clearance of H_2O_2 was monitored using 240 nm wavelength at 25°C. Catalase activity was expressed as in mmol of H_2O_2 consumed/min.

Determination of Acetylcholinesterase Activity

Acetylcholinesterase activity was monitored following the method described by Ellman et al., [27]. The reaction mixture contain 285 µl of distilled water, 180 µl of 100 mM potassium phosphate buffer (pH7.4), 60 µl of 10 mM DTNB, and 15 µl of sample, 60 µl of 8 mM acetylthiocholine were added. The change in absorbance was monitored at 412 nm for 2 min at 10 s intervals, using a UV Spectrophotometer. The enzyme activity was expressed as micromole/min/mg of protein.

Molecular docking studies

The crystal structure of acetylcholinesterase was obtained from the protein database file 4EXY. The file was prepared by extracting solvent molecules, co-crystallized ligands (donepezil) and configured using Chimera v 1.1 to model physiological conditions [28]. Added polar hydrogen and allocated partial charges to the regular residue using Gasteiger partial charge means all the hydrogen atoms are specifically represented. However, the most favorable binding interactions were ascertained using AutoDock Vina. The docking complex interactions were analyzed visually using Discovery Studio 2017 R2 Client (v17.2.0.16349). AutoDock Vina uses conformation-dependent algorithms to rate interactions binding ligands:

$$c = \sum_{i<j}^{n} f_{titj}\ (r_{ij}) \qquad (1)$$

In silico pharmacokinetic and toxicity studies

Some ADMET properties of quercetin was evaluated using ADMETlab platform

(http:/admet.scbdd.com/webserver/ADMETprediction) as defined by [29] Jie et al. (2018). The physicochemical properties, distribution, and toxicity evaluation was performed using the canonical SMILE format of the respective compounds derived from the PubChem database. The study is based on a thorough exploration of a systematic database of 288,967 entries from different sources, including peer-reviewed journals, ChEMBL, EPA, and DrugBank databases. All data were divided into six groups (basic, A, D, M, E, and T) and a set of subclasses by their endpoint meanings. Molecular Operating Environment (MOE, version 2016) has tested and evaluated the corresponding necessary details and experimental values of these entries, forming the basis for predicting a new compound based on computational similarity check.

Statistical analysis

The findings were shown as mean ± SEM. GraphPad Prism 5 software was used for Statistical analysis. The disparity in treatment groups was examined using One way ANOVA, and the difference was considered significant at $P<0.05$. Microsoft Office Excel 2007 used to plot graphs.

Results

Antioxidant biomarkers

Treatment with lead acetate induced a sharp decrease in total thiol content [Figure 2]. Co-administration of lead acetate and quercetin was able ameliorate the decrease in total thiol content observed in a group treated with lead acetate alone. The activity of glutathione-S-transferase and catalase was also shown to be compromise by administration of lead acetate [Figure 3 and 4]. Quercetin alone as well as in co-administration with lead acetate improve the decline in activity of both glutathione-S-transferase and catalase.

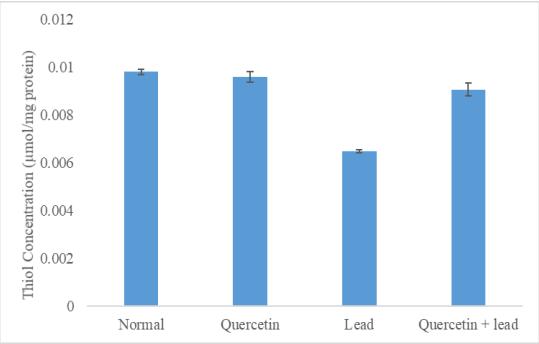

Figure 2. Effects of Quercetin on Total Thiol Concentration in Lead Treated Drosophila.* = significant is when compared to normal control group.

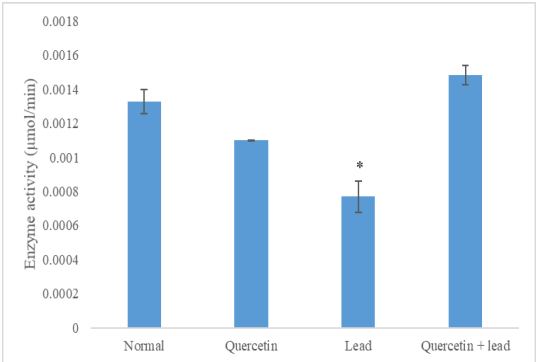

Figure 3.Effects of Quercetin on Glutathione-S-transferase Activity in Lead Treated Drosophila Melanogaster. * = difference significant when compared to normal control group.

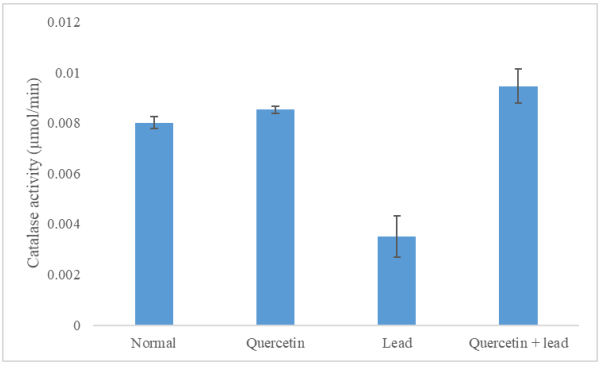

Figure 4. Effects of Quercetin on Catalase Activity in Lead Treated Drosophila Melanogaster.
*** = difference significant when compared to normal control group.**

Acetylcholinesterase activity

The activity of acetylcholinesterase was drastically reduce due to lead acetate exposure, while co-administration with quercetin resuscitate its activity to optimum [Figure 5]. The activity of the enzyme has also improved after administration of quercetin alone.

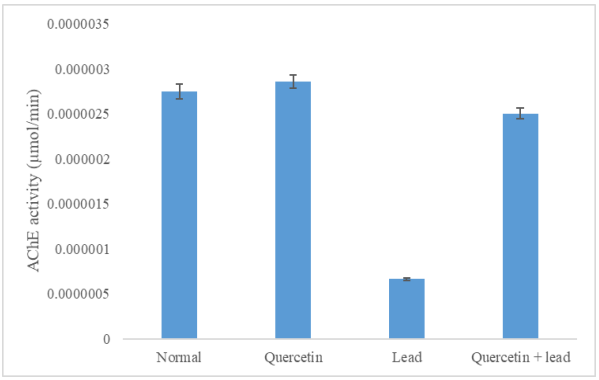

Figure 5.Effects of Quercetin on Acetylcholinesterase Activity in Lead Treated Drosophila Melanogaster. * = difference significant when compared to normal control group.

***In silico* Pharmacokinetic and Molecular Docking Studies**

Pharmacokinetic showed quercetin to confer moderate aqueous and non-polar solubility, high therapeutic index, non-mutageni c and hepatotoxic properties as depicted in Table 2. It also demonstrated excellent permeability based on epithelial colorectal adenocarcinoma cell-line (caco2-), while compound could remain in the body due to their low clearance rate. Molecular docking studies reveal very motivating binding energy and inhibition constants of different interactions, as shown in Table 3. The interactions included the polar and non-polar amino acid residue present in the acetylcholinesterase binding pockets [Figure 6-8]. Furthermore, there were hydrogen, hydrophobic, and other non-conventional interfaces between the compounds and the intended receptor.

Table 2. *In silico* Toxicity and Pharmacokinetic Prediction Profile on Quercetin and its.

Category	Property (unit)	Predicted Result		Inference / Reference Range
		QCN	**DPL**	
Basic physicochemical property	LogP (partition coefficient) (log mol/L)	1.99	2.59	LogP <0: poor lipid bilayer permeability. LogP >3: poor aqueous solubility.
	LogD7.4 (Distribution coefficient D) (log mol/L)	0.14	0.79	<1: High Solubility; 1 to 3: Moderate Solubility; ≥3: Low Solubility.
Absorption	Papp (Caco-2 permeability) (cm/s)	-6.17	-5.12	Optimal: higher than -5.15 or -4.70
Distribution	PPB (Plasma protein binding) (%)	94.9	87.3	90%: Significant with drugs that are highly protein-bound and have a low therapeutic index.
	BBB (Blood brain barrier) (%)	0.24	0.17	≥0.1: BBB positive. <0.1: BBB negative.
Excretion	Clearance (mL/min/kg)	2.05	1.89	Range: >15 high; 5< Cl <15: moderate; <5: low.
	T1/2 (Half life) (H)	0.20	1.08	Range: >8H: high; 3h< Cl <8H: moderate; <3H: low.
Toxicity	H-HT (Human Hepatotoxicity)	0.56	0.64	>0.5: HHT positive <0.5: HHT negative
	AMES (Ames mutagenicity)	0.74	0.08	>0.5: Positive <0.5: Negative

QCN = Quercetin and DPL = Donepezil

Table 3. Binding Energy and inhibition binding Constant of Quercetin against Acetylcholinesterase Receptor bound to Donepezil (4EXY).

Quercetin	Binding Energy (µM)	Inhibition Binding Constant(kcal/mol)
Quercetin	-10.1	0.981
Donepezil	-10.6	0.981

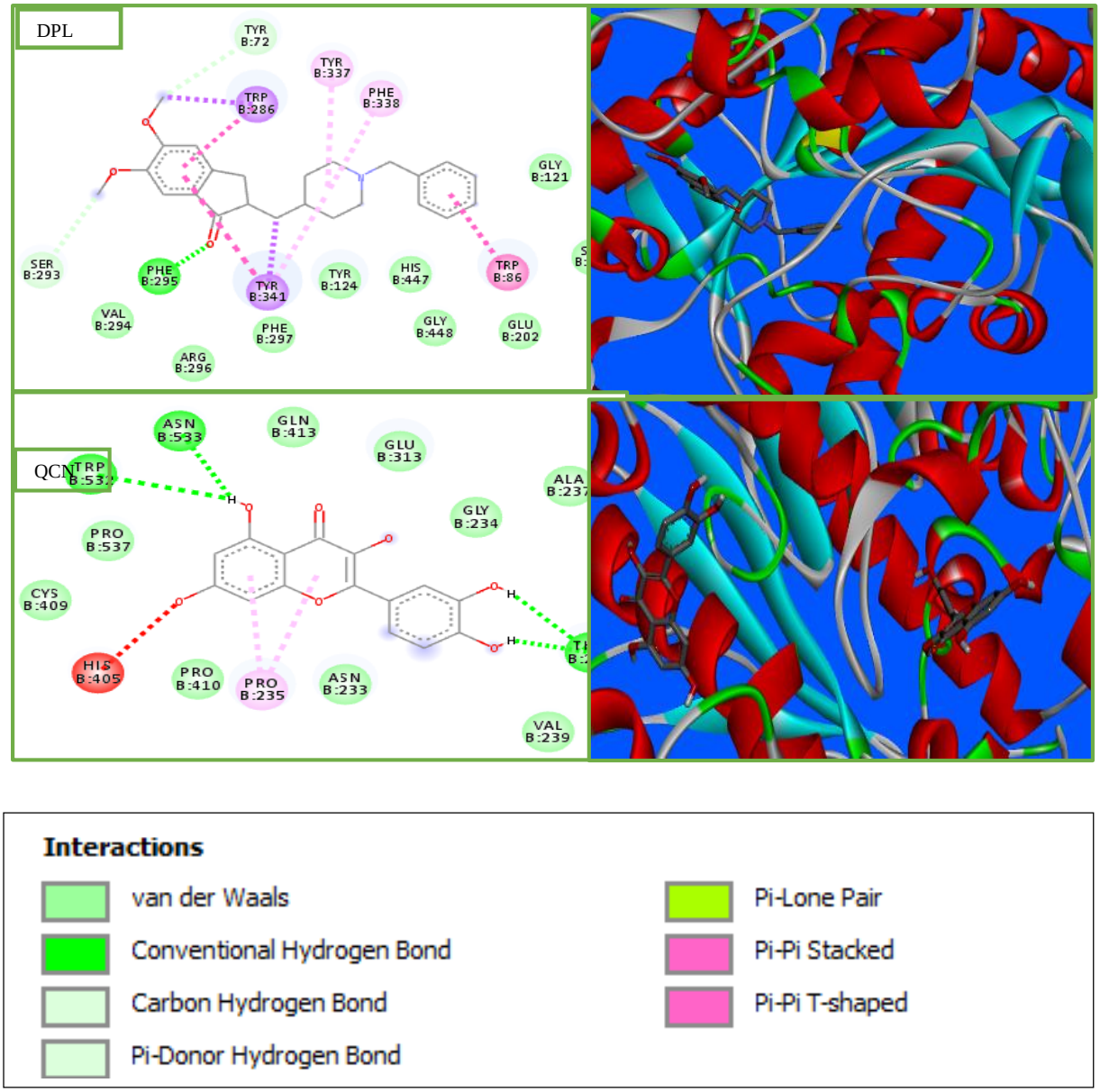

Figure 8. Amino acids interactions and 3D conformations of quercetin within the binding pocket of acetylcholinesterase. QCN = Quercetin, DPL = Donepezil.

Discussions

The function of oxidative stress and cholinesterase activity is one of the most verified hypotheses and try to explain the pathophysiology associated with neurodegeneration [30,31]. Oxidative radicals have been documented to play a critical role in promoting cellular damage, particularly in fatty tissues, and have been involved in neurodegenerative disease pathogenesis [32]. Intensifying evidence indicates that oxidative radicals may play a crucial role in the brain of patients with neurodegenerative diseases [33,34]. Upregulation of antioxidant biomolecules due to administration of quercetin observed in this study could be explained from two viewpoint. The first point could be linked to quercetin ability as an excellent antioxidant/metal chelator to donate a proton thereby quenching oxidative chain reaction [35]. And the second reason is that quercetin might possess the ability to induce gene expression for the production of catalase, thiol and glutathione-S-transferase. Hydroxyl, carbonyl groups and resonance activity in flavonoids and other phytocompounds were detailed to be responsible for their antioxidant and chelation properties [36, 37]. Marija et al., [11] asserted that in most cases the capacity for antioxidants is directly proportional to the number of free hydroxyl groups.

Acetylcholinesterase (AChE) is an enzyme of the α/β hydrolase-fold superfamily with a critical role in synaptic neurotransmission [38, 39]. It is responsible for terminating the nerve impulses in cholinergic and neuromuscular synapses by separating the acetylcholine neurotransmitter into choline and acetate [40, 41]. This influence results in downstream

modulation of striatal and hippocampal neurons of significance to motion, learning, and memory [(42)]. Hence given the aforementioned global advocacy for expanded use of nutraceuticals in managing various diseases, including neurodegenerative disorders, this study was conducted to highlight the efficacy of certain food-based phytocompounds; with Alzheimer's disease, the activity level of AChE enzyme declines and the level of butyrylcholinesterase activity increases in the brain [(3)]. Dual inhibition strategy on these enzymes has been reported most recently to increase the effectiveness of the treatment rather than to restore the initial balance. In this attempt, like in many current approach, we attempt to focus on restoration of the initial balance between acetylcholinesterase and butyrylcholinesterase by reactivation of acetylcholinesterase activity. Finding in this work revealed quercetin to confer the potentials to induce the activity of acetylcholinesterase. This finding is in agreement with many works reported in the earlier studies [(43)]. Ulrike et al., [(44)] affirmed that development of bivalent ligands that occupy both the active and the peripheral site of acetylcholinesterase might be more beneficial for treatment of Alzheimer´s disease than simple inhibition of the acetylcholine hydrolysis. Molecular docking studies revealed the structures of quercetin could be potential acetylcholinesterase reactivators based on their binding site and activation constant. The activation binding process was validated by identifying the co-crystallized AChE inhibitor donepezil. Donepezil bind to acetylcholinesterase through different kinds of interactions which essentially involved Phe295 and many other amino acid residues such as Trp286, Trp341, Trp81, Val294, Gly121, Arg296, Phe297, Tyr124, His447, Gly448, Glu202 and Ser203, respectively. Quercetin bind to acetylcholinesterase through various amino acid residue including those involved in its interaction with donepezil. Omamuyovwi and Augustine [38], shows Kolaviron may be a novel herbal, medicinal product to treat neurodegenerative disorders associated with dysregulated cholinergic neurotransmitter systems. These compounds were further predicted to safe and physicochemically promising bioactive molecules. This finding correlate with the work reported by Aliyu et al.,[(45)] , who predicted a great Pharmacokinetic potentials of some phytocompounds. Interestingly, quercetin could be transferred efficiently to their different targets via the bloodstream as proof of plasma-binding abilities.

Conclusion

Based on the present work, quercetin shows potentials ability to restore acetylcholinesterase activity in lead induced damage in drosophila melanogaster species. It's further demonstrated the ability of up regulate the production of biological antioxidant molecules. By correlating the docking outcomes with experimental data obtained, it can be suggested that presence of quercetin alongside many other phytocompounds in food could be reasonable in the management of Alzheimer's diseases and related neurodegenerative disorders. Further studies should be performed on other phytocompounds for the treatment of neurodegenerative disorders.

Declaration of interest

The authors declared no competing interest with regards to the publication of this paper.

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