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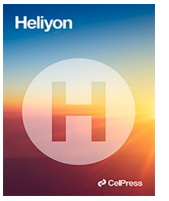

Research article

Divine noni's protective impact on Swiss albino mice's short-term memory impairment caused by cyclophosphamide: A behavioral and biochemical approach

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ABSTRACT

Cyclophosphamide (CYL) is a first-line cancer chemotherapeutic agent widely used for the treatment of cancer that has severe toxic effects. The primary mechanism by which CYL induces toxicity through free radical generation. *Morinda citrifolia* (Noni) fruit juice is an herbal remedy documented to have antioxidant properties. The aim of the current study was to investigate the protective effect of noni against CYL-induced memory impairment in Swiss albino mice. Treatment schedule: Group 1: Normal: Received vehicle; Group 2: CYL treatment: Received CYL (40.0 mg/kg b.w. i.p.) on day one; Group 3: NJ treatment: Received NJ (360 mg/b.w. p.o.) once daily for 14 days. Group 4: DNG treatment: DNG (360 mg/b.w. p.o.) once daily for 14 days, Group 5: NJ + CYL treatment: Received CYL (40.0 mg/kg b.w. i.p.) on day one and after half an hour of received NJ (360 mg/b.w. p.o.) once daily for 14 days. Group 6: DNG + CYL treatment: Received CYL (40.0 mg/kg b.w. i.p.) on day one and after half an hour received DNG (360 mg/b.w. p.o.) once daily for 14 days. Mice were subjected to the Morris water maze (MWM) challenge for two weeks as part of a behavioral study. Short-term memory impairment was observed in the behavioral activity of CYL-treated mice in the MWM test in the 1st week trial, and this effect was

Abbreviations: CYL-Cyclophosphamide, MDA-Malondialdehyde; MI-Memory impairment, AChE-Acetylcholinesterase; CAT-Catalase, SOD-Superoxide dismutase; GSH-Glutathione, GST-Glutathione S-transferase; MWM-Morris water maze, ROS-Reactive oxygen species; TSTQ-Time spent in target quadrant, ELT-Escape latency time; NJ-Noni juice, DNG-Divine noni gold.

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reversed in the 2nd week trial in the combination treatment group. The behavioral analysis proved that noni supplementation reduced the risk of memory impairment caused by CYL. Biochemical analysis revealed that CYL markedly increased the levels of AChE and MDA in brain tissue. Similarly, decreases in the levels of antioxidants, i.e., GSH, CAT, SOD and GST, were detected in the brain tissue of the mice exposed to CYL. Qualitative and quantitative examinations of histopathological examination of the mouse hippocampus supported the above findings. The results demonstrated that noni supplement therapy reversed the changes in the MDA, AChE, and antioxidant enzyme levels while improving the behavioral and histological alterations caused by CYL. Long-term hippocampal growth and memory are unaffected, suggesting that CYL is less harmful. According to our research, supplementing with noni in conjunction with CYL may be a helpful treatment strategy for treating memory impairment caused by CYL.

1. Introduction

Cyclophosphamide (CYL), a well-known anticancer medication, is used to improve the prognosis of cancer patients. Despite its ability to cure cancer, CYL is also linked to several undesirable side effects because of its metabolites, such as phosphoramide mustard and acrolein [1]. Several studies have reported that CYL can cause various toxic effects, including nephrotoxicity, hepatotoxicity, neurotoxicity cardiotoxicity etc. [2–5]. Extremely reactive oxygen species (ROS) are produced by lipid peroxidation, which is caused by the CYL metabolite acrolein. When these plentiful ROS interact with another cell, cellular damage can occur [6,7]. It is well known that CYL and its metabolites are neurotoxic [8–11]. By reducing the increase in ROS levels, it is possible to diminish the toxic effects of CYL. Phytochemical compounds with antioxidant properties have been revealed to protect cells from CYL-induced oxidative imbalance [12]. Furthermore, hippocampal neurogenesis has been reported to be unfavorably linked to memory impairment (MI) [13]. Hippocampal neurogenesis is thus seen as a crucial component of cognitive function. Chronic stress also inhibits hippocampal development because it increases the level of oxidative stress. Frequent restraint stress has been shown to increase the blood levels of glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) in rats [14]. According to another study, malondialdehyde (MDA) levels in mouse brains could be increased by persistent stress [15].

Reported that natural antioxidant consumption reduces oxidative stress and enhances cognitive function [16]. Notably, the combination of wheel-running exercise and phytoconstituents such as polyphenols, flavonoids, and epicatechin increased the preservation of spatial memory. According to reports, strawberry fruit extract can guard against spatial memory deficiencies caused by whole-body radiation [17,18]. Tissue antioxidants are the first line of defense against free radical toxicity. The equilibrium of oxidation–reduction between pro-oxidants and antioxidants is crucial for regular cellular functioning [19].

Both superoxide dismutase (SOD) and CAT plays crucial role in cellular stress; SOD scavenges superoxide radicals by altering them to peroxide and molecular components [20], whereas CAT reduces peroxide and protects normal cells from extremely reactive hydroxyl radicals [21]. It has been reported that GliSODin, a SOD extracted from melons and combined with gliadin, prevents stress-induced impairment of cognitive function and maintains neurogenesis in the hippocampus [22]. Umukoro et al. reported that excessive acetylcholine (ACh) release and activation of the inflammatory cascade result in behavioral alterations upon social defeat stress induction. Nonetheless, the presence of naringenin has been shown to reduce elevated concentrations of IL-1β, MDA, and acetylcholinesterase (AChE) in brain tissues [23].

Morinda citrifolia is an herbal plant native to the Pacific Islands that is commercially called noni and is used as folk medicine by Polynesians. Noni fruit juice possesses antioxidative activity [24]. The methanolic extracts and ethyl acetate-soluble phases of Noni fruit have been shown to prevent the effects of copper on low-density lipoprotein (LDL) oxidation [25]. Numerous phytochemical compounds, such as gallic acid, ascorbic acid, rosmarinic acid, ellagic acid, chlorogenic acid, epigallocatechin gallate, quercetin, (R)-naringenin, ascorbic acid, isorhamnetin, ellagic acid, and ursolic acid, are found in noni fruit [26,27]. Due to the presence of many of these phytochemicals in noni fruit, it is known to be an antioxidant and anti-inflammatory agent [28].

Various other biological activities, such as antimicrobial, antibacterial, antifungal, and anticancer activities, have also been reported in *M. citrifolia* [29–34]. Fresh noni fruit juice (NJ) is very well liked worldwide because of its pharmacological and nutritional properties. One product on the market named Divine Noni Gold (DNG), which comprises fresh NJ, *Garcinia cambogia* extract, and *Glycyrrhiza glabra* root extract. *Garcinia cambogia* is a plant that belongs to the *Clasiaceae* (*Guttiferae*) family and has a wide range of therapeutic benefits. Many phytochemicals, including garcinol, isogarcinol, and xanthone compounds such as oxyguttiferone M, oxyguttiferone K, oxyguttiferone K2, and oxyguttiferone I, have been shown to be present in *Garcinia cambogia* fruit. These compounds exhibited antioxidant potential [35]. The presence of polyphenols, among which flavonoids, coumarins, anthroquinones, and phenolic compounds are primarily present in *Morinda citrifolia* and are attributed to their antioxidant activity, is confirmed by the presence of polymeric hydroxyl groups, aromatic compounds, phenols, and aldo-keto group peaks [35]. The literature reported that noni fruit extracts dose-dependently inhibit oxidative substances caused by lipid hydroperoxide [36]. Jianguo et al., studied on neuroprotective activity of chloroform and aqueous fractions of noni juice against t-Butyl hydroperoxide-caused oxidative damage in SH-SY5Y cells. Suggested that the antioxidant and anti-inflammatory properties of noni juice can offer a protective effect against oxidative stress-induced neurodegenerative diseases due to presence of many bioactive phytochemical constituents, i.e. glycosides, iridoids, anthraquinones, flavonoids, phenolic acid, and coumarins [37].

The protective effect of noni on CYL related oxidative stress-induced MI have not been reported in the literature. Therefore, the present study aims explore whether noni (NJ and DNG) adjuvant therapy could diminish against CYL related oxidative stress-induced

MI and whether noni could improve cognitive function associated with neurogenesis in the hippocampus.

2. Materials and methods

2.1. Materials

Dimethyl sulfoxide (DMSO), fetal bovine serum (FBS-Gibco), and Dulbecco's modified Eagle's medium were obtained from DMEM-HiMedia Laboratories (Mumbai, India). Sulfo-Rhodamine-B (SRB) and secondary antibodies that were HP-conjugated with horseradish peroxidase (CAS number 9003-99-0) were purchased from Sigma Aldrich (Mumbai, India). Primary antibodies (goat polyclonal anti-human Nrf2 (CAS number 75483-04-4) and internal control actin [(CAS 51005-14-2) in TBS] were used in combination with phosphate-buffered saline (PBS), bovine serum albumin (BSA; CAS number 9048-46-8), tris-buffered saline (TBS), and enhanced chemiluminescence substrate (ECL) from Thermo Fisher Scientific (Mumbai, India). NQO1 lysis buffer was obtained from Santa Cruz Biotechnology (Mumbai, India). MDA, SOD, GSH, and Glutathione S-transferase (GST) assay kits were purchased from IBL International (Bangalore, India). Ripe noni fruit and DNG were procured from Noni Biotech Pvt. Ltd. (Tamil Nadu, India). Commercially available CYCLOXAN 500 mg (Cyclophosphamide injection IP 500 mg) was obtained from the Medical Store of JSS Hospital, Mysore, India.

2.2. Noni products

Fresh Noni Juice (NJ) and Divine Noni Gold (DNG) juice were used as test samples. DNG is a marketed product that contains dried extract of noni fruit, *Garcinia cambogia* extract, and *Glycyrrhiza glabra* root extract. Fresh NJ was produced entirely from cleaved noni fruit by a hand press. Noni products were kept in a refrigerator throughout the experiment.

2.3. Fourier transform infrared (FT-IR) analysis

The Jagmohan (2005) [38] approach has been applied to FTIR studies. In an evacuated die, a potassium bromide (KBr) pellet was combined with the powdered fruit (5 mg) sample to create a clear, translucent disc with dimensions of 13 mm in diameter and 1 mm in thickness. On a PerkinElmer Fourier transform spectrometer (L1600235, PerkinElmer, Akron, OH, USA) with an air-cooled deuterated triglycine sulfate (DTGs) detector, FTIR spectra in the 4000-400 cm-1 frequency range were acquired at room temperature. One hundred scans with a spectral resolution of 4 cm were combined for each spectrum. The frequencies of all the sharp bands were within 0.01 cm. The (%) transmittance unit was used to express all spectral values.

2.4. Identification and quantification of bioactive compounds using LC–MS/MS analysis

The DNG was analyzed through liquid chromatography–mass spectrometry (LC–MS/MS) [39–41]. The instrument used was a SYNAPT-XS HDMS, UK (UPLC ACQUITY H CLASS Series System, DBA064). C18 Waters, Acquity BEH (2.1*100 mm, 1.7 μm), 5 μL injection volume, 40 °C column temperature and 0.3 mL/min were used. The mobile phase comprised 0.1 % formic acid + LC–MS grade water (phase A) and 0.1 % formic acid + acetonitrile (phase B). The eluent was directly routed to the electrospray ionization (ESI) module. The gradient details are given below (Table 1).

2.4.1. Acquisition mode

Spectra were recorded in positive ionization mode between *m/z* 150 and 2000.

2.5. Animals

Animal maintenance was performed according to the WHO guidelines (Geneva, Switzerland). The inherited Swiss albino mice were obtained from the JSS Medical College, Mysuru, India, for *in-vivo* studies. Fifty-to seventy-day-old mice of 24–30 g of weight was designated as well-maintained in controlled environments of humidity (50 ± 5 %) and temperature (23 ± 2 °C), and the light was maintained at 14 h and 10 h of light and dark, respectively, at the Central Animal Research Facility JSS College of Pharmacy, Mysuru, India [42]. The mice were provided hygienic food and water ad libitum. Mice in different clusters were placed in polypropylene cages

Table 1
Gradients used.

Time (min)	Flow (μL/min)	%A	%B	Curve
0.00	0.300	95.0	5.0	6
1.00	0.300	95.0	5.0	6
07.00	0.300	30.0	70.0	6
08.00	0.300	10.0	90.0	6
10.00	0.300	10.0	5.0	6
11.00	0.300	95.0	5.0	6
15.00	0.300	95.0	5.0	6

with sterile paddy husks for bedding. The study was approved by the Institutional Animal Ethical Committee (IAEC No. 162/2016) of the JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, India.

2.6. Experimental design

Thirty-six mice were assigned to the following clusters. Each cluster contained six mice. Group 1: Control group, consumed drinking water; group 2: received CYL (40.0 mg/kg b.w. i.p.) on day 1; group 3: received NJ (360 mg/b.w. p.o.) once daily for 14 days, group 4: received DNG (360 mg/b.w. p.o.) one time daily for 14 days, group 5 received CYL (40.0 mg/kg b.w. i.p.) on day 1 and received NJ (360 mg/b.w. p.o.) one time daily for 14 days, and group 6: received CYL (40.0 mg/kg b.w. i.p.) on day 1 and received DNG (360 mg/b.w. p.o.) once daily for 14 days. CYL was used as a positive control. Sector-specific safety testing criteria differ, but generally, repeat-dose testing in animals serves as the foundation for evaluating the risks to human health. The ideas outlined in a technical study by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), which suggests practical methods for dose selection considering legal requirements, animal welfare, and cutting-edge scientific methodologies, are further expanded upon in this work. NJ and DNG (0.35 ml) were calculated from the human dose to the mouse dose. A total of 0.35 ml is equivalent to 360 mg measured by a digital weighing balance. A repeated dose toxicity assay (28 days) for the ethanolic extract of *M. citrifolia* fruit in rats has already been reported. According to reports, repeated administration of the said extract at doses of 500, 1000, and 2000 mg/kg body weight over the course of 28 days did not result in any appreciable changes to the body weight, hematological, or biochemical markers in the animals. In addition, histopathological examination of different organs did not reveal any morphological alterations. Hence, the noni fruit was found to be safe. Because it is reported to be safe, a toxicity test was not performed in this study [43].

2.7. Behavioral assessments

2.7.1. Morris water maze method

In each experiment, mice in each group were trained in the Morris water maze (MWM) daily for 14 days before treatment [44]. Training was given by placing the mice in the water on the side of the wall facing toward the wall of the MWM. Throughout the training period, each mouse underwent 3 trials/day with 5-min intertrial intervals for 14 days or until the mice met the required criteria. After 14 days of training, the mice were randomized into different groups, and basal readings were taken on day 15. After half an hour of basal reading, the mice of each group were treated as per the experimental design mentioned above, and behavioral performance was assessed on the 7th and 14th days of the two-week treatment in the MWM with a cutoff period of 60 s. The platform in the MWM was kept slightly below the water level, and the same position was maintained throughout the escape latency time (ELT) study.

The initial measure of learning is escape latency, which is the time required to find the platform, called ELT. In the present study the ELT was performed by placing the mice in the water on the side of the wall facing toward the wall of the MWM and noting the time to reach each mouse on the platform. The platform was removed from the MWM, and the mouse was placed on the water in the same manner next to the wall to measure the amount of time spent in the target quadrant (TSTQ) [45].

2.7.2. Sample collection

Following the behavioral study, all the mice in the groups were sacrificed on the 15th day. Brain tissues were isolated, cut into two pieces, and then quickly washed with normal saline. For histological analyses, a piece of the brain was kept in 10 % phosphate-buffered formalin. Another piece of the brain was used for biochemical assessments and was homogenized at 1/10 w/v in ice-cold Tris-HCl buffer (0.1 M, pH 7.4) [46].

2.7.3. Biochemical measurements

Using a Denville Ultra EZ grind tissue homogenizer, equivalent amounts of brain tissue were homogenized for 5 min in 0.1 M cold potassium phosphate buffer (pH 7.4). To extract the supernatant, the homogenate was centrifuged at 14,000 rpm for 15 min at 4 °C, after which the concentrations of MDA, GSH, CAT, SOD, GST, and AChE were measured using a Shimadzu spectrophotometer (UV120-02) in accordance with each enzyme's usual protocol [47–49].

2.7.4. Histopathological investigation

The hippocampal tissue was transferred to 10 % formalin for 24 h and then fixed in paraffin blocks, and 5 μm thick slices were prepared from the blocks and stained with hematoxylin and eosin dye. The prepared slides were focused under a phase contrast microscope (OLYMPUS CKX41). Images were captured for analysis of cellular architecture in different treatment groups [50]. The quantitative histological assessment of NJ and DNG in the brain hippocampus of CYL-challenged mice was carried out for 7th and 14th days.

2.8. Statistical analysis

Data analysis was performed with GraphPad Prism version six. The data are presented as the mean ± SEM of one-way and two-way analysis of variance (ANOVA) tests, followed by Tukey's multiple comparisons test and p value was evaluated.

3. Results

3.1. FT-IR analysis

FTIR spectroscopy is a helpful method for describing and identifying chemicals or functional groups (chemical bonds) present in an unidentified combination of DNG. Furthermore, functional group characterization and identification using FTIR spectroscopy is a well-established approach that saves time [51].

The FTIR results supported the presence of alcohols, carboxylic acids and amines (3253.499 cm^{-1}), alkynes (2118.195 cm^{-1}), amides and alkenes (1639.191 cm^{-1}), nitro and alkanes (1417.573 cm^{-1}), alcohols, esters, ethers, carboxylic acids, anhydrides and amines (1174.183 cm^{-1}), amines and fluorides (1083.529 cm^{-1}), tertiary alcohols, and fluorides (1019.440 cm^{-1}) (Fig. 1 and Table 2).

3.2. LC–MS analysis of the divine noni gold (DNG)

3.2.1. Total ion chromatogram (positive mode)

LC–MS analysis of the DNG revealed the presence of several compounds. In the extract, a total of 18 compounds were identified in positive mode, as mentioned in Figs. 2 and 3 and Table 3.

3.3. Behavioral examinations

3.3.1. Defensive effect of the NJ and DNG on CYL-Induced short-term memory deficits in Swiss albino mice: A behavioral and biochemical approach

3.3.1.1. Determination of ELT. Compared with the control, a single dose of CYL at 40 mg/kg b.w. significantly (**$p < 0.01$) augmented ELT in the 7th and 14th day trials. In 14th day trial we have observed CYL treated group were taken short time (7.94 ± 1.22) to reach in platform compared to 7th day trial (8.95 ± 0.73). Result indicates that mice were getting recover MI in 14th day, suggests that CYL induce short term memory. Supplement therapy with NJ and DNG significantly (*$p < 0.05$) reduced the ELT in combination therapy, i.e., CYL + NJ and CYL + DNG. However, individual NJ and DNG therapies-maintained ELTs close to control. However, DNG + CYL (**$p < 0.01$) showed more significant effect than NJ + CYL (*$p < 0.05$) in 14th day trial (Fig. 4).

3.3.1.2. Determination of TSTQ. The administration of CYL leads to significant (*$p < 0.05$) declined TSTQ when compared to control in day 7th and 14th. In 14th day trial mice spent more time (24.0 ± 4.4) in the platform area than 7th day (21.28 ± 5.36) trial. Here also results indicates that mice were getting recover from MI. Individual treatment with NJ and DNG significantly augmented TSTQ in comparison to treatment with CYL alone on both the 7th (**$p < 0.01$) and 14th (***$p < 0.001$) days of the trial. Compared with CYL treatment alone, combination therapy with CYL + DNG significantly (*$p < 0.05$) reduced the TSTQ on the 14th day, but the difference was not significant on the 7th day. However, CYL + NJ did not have a significant effect on either the 7th or 14th day of the trial (Fig. 5). Both the ELT and TSTQ findings indicate that CYL induces MI, which can be averted or minimized by supplement therapy of noni with CYL.

3.3.2. Biochemical measurements

3.3.2.1. Effect of noni on the CYL-induced oxidative variance in brain tissue. In the control, the antioxidant enzyme i.e. GSH, SOD, CAT

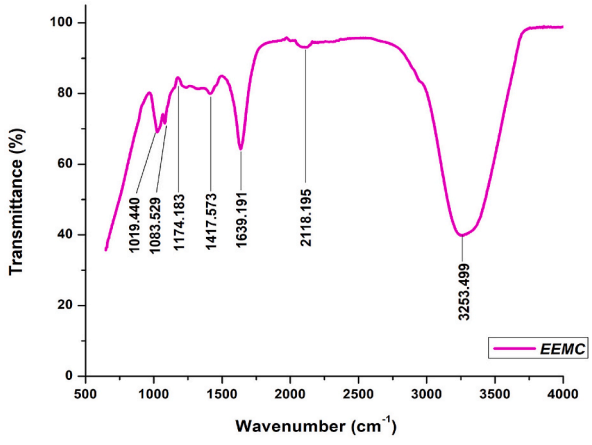

Fig. 1. FTIR spectra of divine noni gold (DNG).

Table 2
FTIR peak analysis and functional groups present in the divine noni gold (DNG).

S. No.	Wavenumber (cm^{-1})	Compound class	Functional group
1	3253.499	Alcohol	O-H stretching
		Carboxylic acid	O-H stretching
		Amine	N-H stretching
2	2118.195	Alkyne	C=C stretching
3	1639.191	Amide	C=C stretching
		Alkene	C=C stretching
4	1417.573	Nitro	N=O bending
		Alkane	C-H bending
5	1174.183	Alcohols, esters, ethers, carboxylic acids, anhydrides	C-O stretching
		Amines	C-N stretching
6	1083.529	Amines	C-N stretching
		Fluorides	C-X stretching
7	1019.440	Tertiary alcohol	C-O stretching
		Fluorides	C-X stretching

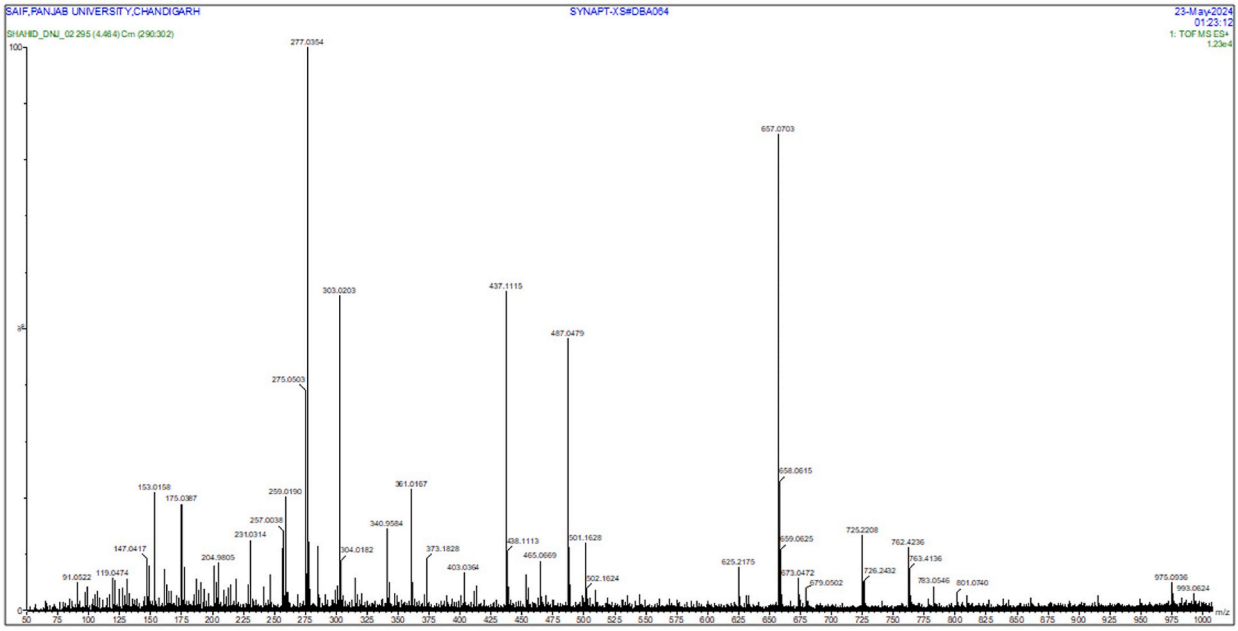

Fig. 2. LC–MS chromatograms of the significant compounds present in DNG.

and GST levels were 10.79 ± 0.72 μmol/mg of protein, 22.15 ± 1.81 units/mg of protein, 81.66 ± 2.56 units/mg of protein and 113.201 ± 1.69 nmol/mg of protein, respectively. Treatment with 40 mg/kg CYL via i.p. injection caused MI, as indicated by significant decrease in the levels of antioxidant enzymes (GSH, SOD, CAT and GST) compared to those in the control group.

Individual treatment with NJ and DNG significantly ($p < 0.05$) improved the GSH, SOD, CAT and GST levels compared to those in the control group, indicating that noni can increase the levels of antioxidant enzymes in healthy organism. The combination treatment of NJ + CYL did not displayed significant difference the levels of SOD, CAT, GST except GSH compared to those in the group treated with CYL alone. However, the combination treatment of DNG + CYL significantly improved the GSH (***$p < 0.001$), SOD (*$p < 0.05$), CAT (***$p < 0.001$) and GST (**$p < 0.01$) levels compared to those in the group treated with CYL alone. The result indicates that CYL treatment can promote oxidative stress in the brain by reducing endogenous antioxidant enzyme levels, and this effect was reversed by 14 days of DNG treatment (Fig. 6).

3.3.2.2. Assessment of MDA and AChE in brain tissue. In the control group, both the MDA and AChE levels were 19.16 ± 0.921 nmol/mg of protein and 137.23 ± 2.79 μmol/mg of protein, respectively. The i.p. injection of 40 mg/kg CYL caused lipid peroxidation in isolated brain tissue and significantly (<0.05) increased the MDA and AChE levels compared to those in the control group. However, individual NJ and DNG treatments significantly ($p < 0.05$) decreased both the MDA and AChE levels compared to those in the control group.

There was no significant difference between the NJ + CYL combination treatment group and the CYL alone treatment group.

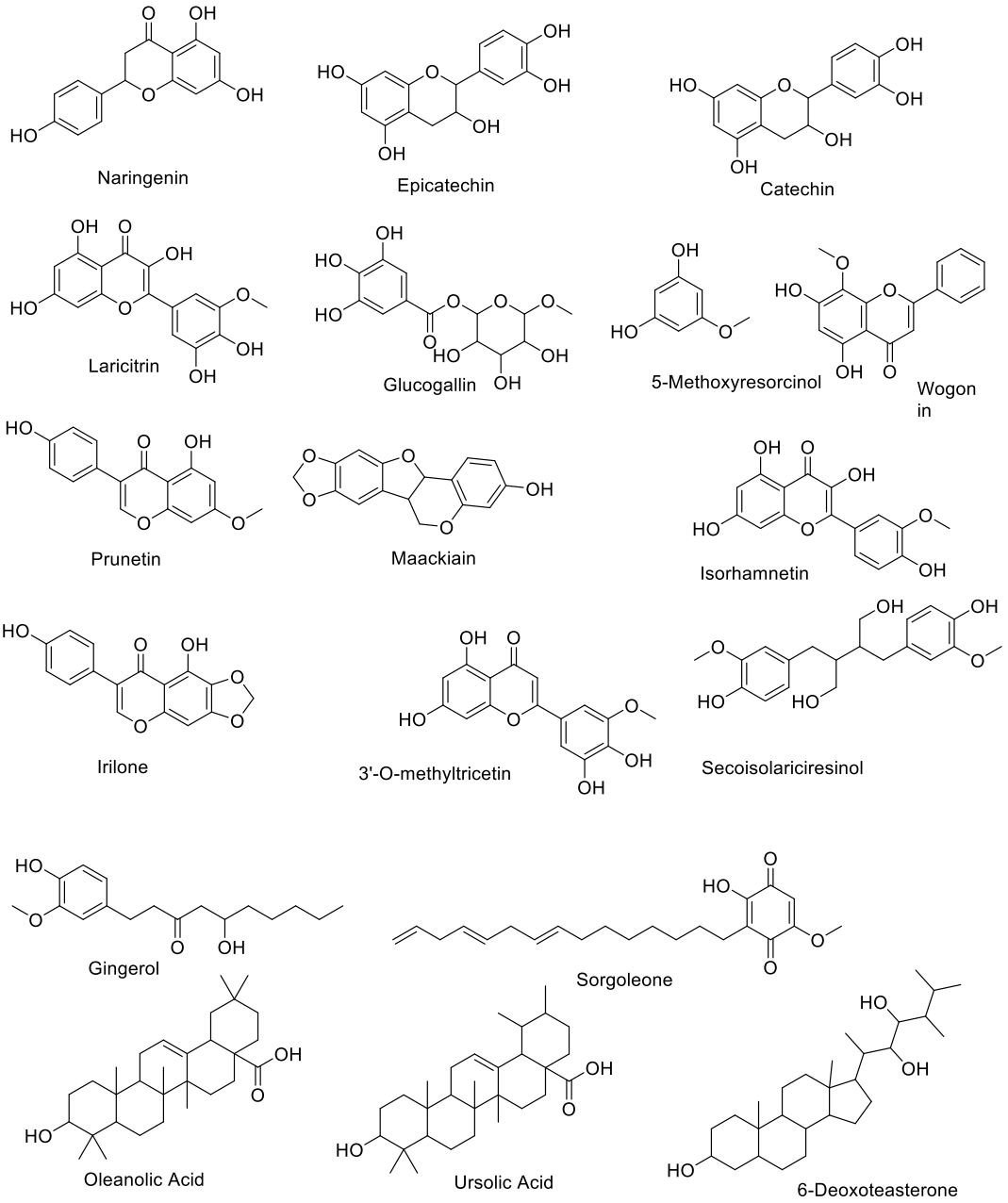

Fig. 3. Structures of the compounds identified based on LC–MS from *the ethanolic extract of M. citrifolia.*

However, the combination treatment of DNG + CYL significantly (<0.05) diminished the MDA and AChE levels compared to those in the group treated with CYL alone. This indicates that DNG can protect hippocampal cells against CYL-induced neuronal death by inducing oxidative stress and inhibiting cholinergic dysfunction (Fig. 7).

3.4. Histopathological examination

The above results were confirmed by histopathological studies (Fig. 8). Fig. 8A shows a healthy hippocampus. H&E staining revealed that the degenerated hippocampal cells in the CA1 and CA3 regions after CYL injection (Fig. 8B) were loosely arranged and exhibited obvious pyknosis, indicating that treatment with CYL induces MI by inducing oxidative stress. The behavioral outcomes of the MWM test showed that CYL injection can induce short-term MI in mice, which can be reversed by supplemental therapy with noni. The histopathological figures for 14th day are only presented in the manuscript (Fig. 8).

However, after treatment with NJ (Fig. 8C) or DNG individually (Fig. 8D), no cell degeneration was observed, and the tissue was

Table 3
LC–MS analysis of the ethanolic extract of *M. citrifolia* (DNG).

S. No	R. Time	Score	Compound Name	Ion	Formula	**Adducts**	Exact Mass	Observed Mass
1	1.04	35.5	(R)-naringenin	Positive	$C_{15}H_{12}O_5$	M-H	272.253	271.06
2	1.07	35.8	(−)-Epicatechin	Positive	$C_{15}H_{14}O_6$	M-H_2O-H	290.268	271.05
3	1.09	35.9	(+)-Catechin	Positive	$C_{15}H_{14}O_6$	M-H_2O-H	290.268	271.0
4	4.41	35.9	Laricitrin	Positive	$C_{16}H_{12}O_8$	M + Na-2H	332.26	353.02
5	4.61	36.3	Glucogallin	Positive	$C_{13}H_{16}O_{10}$	M-H	332.26	331.67
6	5.95	37.9	Flamenol	Positive	$C_7H_8O_3$	M-H_2O-H	140.137	121.09
7	5.97	26.5	Wogonin	Positive	$C_{16}H_{12}O_5$	M-H_2O-H	284.263	265.04
8	5.98	36.5	Prunetin	Positive	$C_{16}H_{12}O_5$	M-H_2O-H	284.26	266.55
9	5.99	36.6	(+)-maackiain	Positive	$C_{16}H_{12}O_5$	M-H_2O-H	284.263	265.77
10	6.20	36.7	Isorhamnetin	Positive	$C_{16}H_{12}O_7$	M-H_2O-H	316.26	297.03
11	6.25	36.3	Irilone	Positive	$C_{16}H_{10}O_6$	M-H	298.24	297.34
12	6.21	36.8	3′-O-methyltricetin	Positive	$C_{16}H_{12}O_7$	M-H_2O-H	316.262	297.77
13	8.23	35.3	Secoisolariciresinol	Positive	$C_{20}H_{26}O_6$	M-H	362.41	361.67
14	8.25	37.6	(±)-Gingerol	Positive	$C_{17}H_{26}O_4$	M-H	294.386	293.7
15	9.32	35.6	Sorgoleone	Positive	$C_{22}H_{30}O_4$	M-H_2O-H	358.471	339.13
16	10.94	34.8	Oleanolic acid	Positive	$C_{30}H_{48}O_3$	M-H	456.70	455.44
17	11.41	33.4	Ursolic acid	Positive	$C_{30}H_{48}O_3$	M-H	456.78	45,589
18	11.77	32.8	6-deoxoteasterone	Positive	$C_{28}H_{50}O_3$	M + Na-2H	434.69	455.69

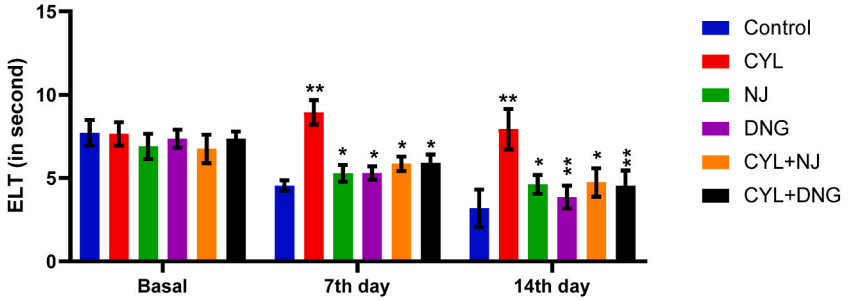

Fig. 4. Effects of NJ and DNG on ELT performance in the MWM test. The values are presented as the mean ± SEM; n = 6. Data was analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. The obtained *p* values were: *p < 0.05; **p < 0.01.

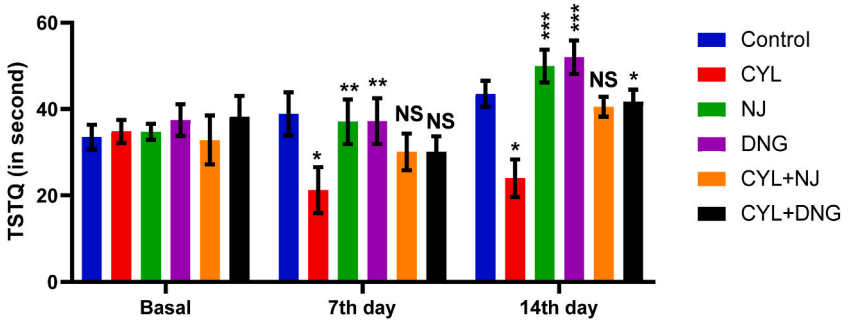

Fig. 5. Effects of NJ and DNG on the TSTQ in the MWM test. The values are presented as the mean ± SEM; n = 6. Data was analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. The obtained *p* values were: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. (NS indicates no significant).

found to be a control section. After the administration of combination treatments, such as NJ + CYL (Fig. 8E) and DNG + CYL (Fig. 8F), NJ and DNG with CYL prevented the degeneration of cells in CA1 and CA3, respectively, indicating that NJ and DNG could prevent the loss of neurons in mice hippocampus.

Cells were counted as the number of cell bodies/mm^2 area of the hippocampus (Fig. 8) and bar graph for quantitative analysis of cells were constructed out of 10 cells (Fig. 9). CYL treated groups showed significant decreased neuron in both 7th (****p < 0.0001) and 14th ($$$p < 0.001) day compared to control group. In combination treated groups number of neurons are significantly improved in both 7th (**p < 0.01) and 14th ($p < 0.05) day compared to CYL alone treated group. Individual treatment of NJ (****p < 0.0001) and DNG ($$$p < 0.001) did not show significant difference compared to control but showed significant difference with CYL alone. Quantitative histopathological assessment of NJ and DNG in the brain hippocampus of CYL-challenged mice for 7 and 14 days revealed

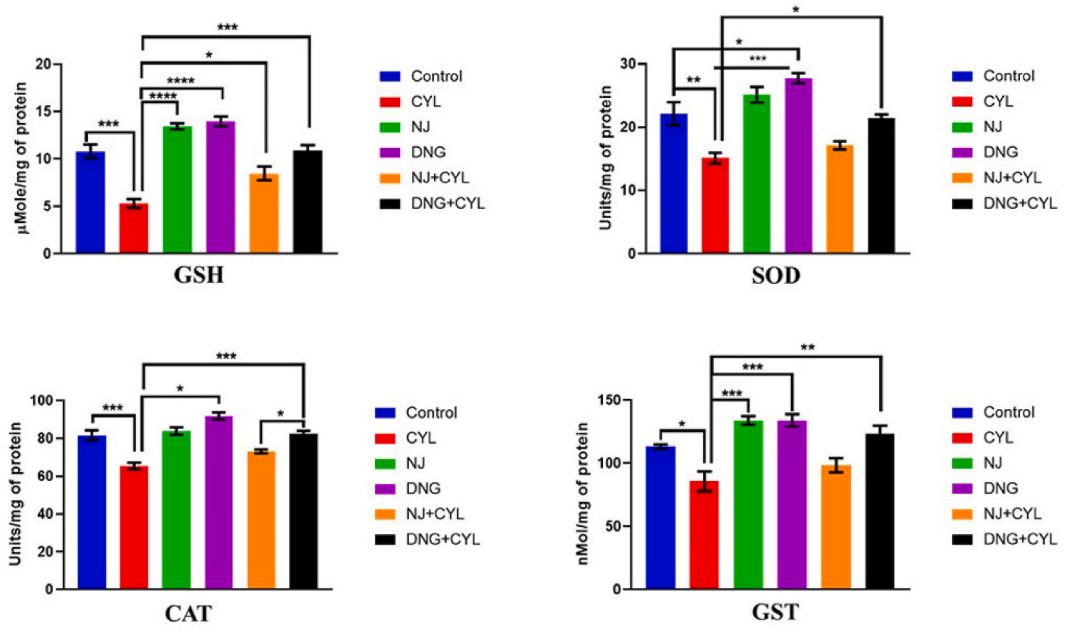

Fig. 6. All the data are presented as the mean ± SEM (n = 6) and were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. The obtained *p* values were: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

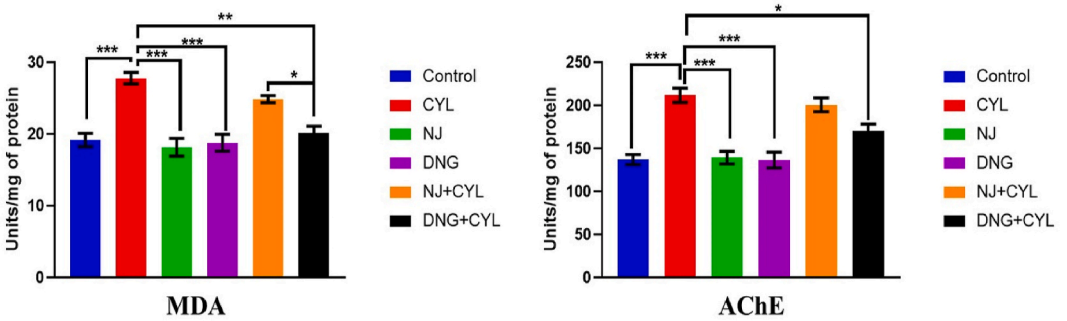

Fig. 7. All the data are presented as the mean ± SEM (n = 6) and data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. Obtained *p* values as follows: *p < 0.05, **p < 0.01, ***p < 0.001.

that NJ and DNG prevented cell degeneration caused by CYL and that the percentage of neurons was reduced minimally, indicating the protective impact of noni.

4. Discussion

To investigate whether the divine noni has any effect on stress-induced impairment of learning and memory, the mice were given CYL, and memory was determined using the MWM test after administration of noni. Numerous phenolic and flavonoid compounds are present in noni fruit and have antioxidant activity [52].

This study aimed to assess the neuroprotective potential of divine noni against CYL-induced MI linked to oxidative stress. The results of the present study are consistent with those of a previous study showing that CYL induces hippocampal toxicity by inducing oxidative stress, which leads to cause MI [53–56]. Clinical studies have demonstrated that cognitive impairment is an adverse effect of cancer chemotherapy that commonly occurs in cancer patients [57,58]. Factors may be defensive in contradiction of cognitive deficits or persons at a greater risk for loss of cognitive role. These factors include linked side effects of cancer and its treatment, such as fatigue and depression, as well as direct and indirect consequences of chemotherapy, such as anemia and/or menopause due to chemotherapy. It has been reported that CYL causes DNA to alkylate and avert the replication of the genome in dividing cells [59].

In the current study, the presence of hydroxyl functional groups in DNG was investigated by FTIR spectrum that may be associated with the various flavonoid and phenolic compounds identified by LC-MS analysis (Fig. 3). Catechins are composed of two benzene rings (A- and B-rings) and a dihydropyran heterocyclic ring (the C-ring) with a hydroxyl group on carbon 3. Functional hydroxyl

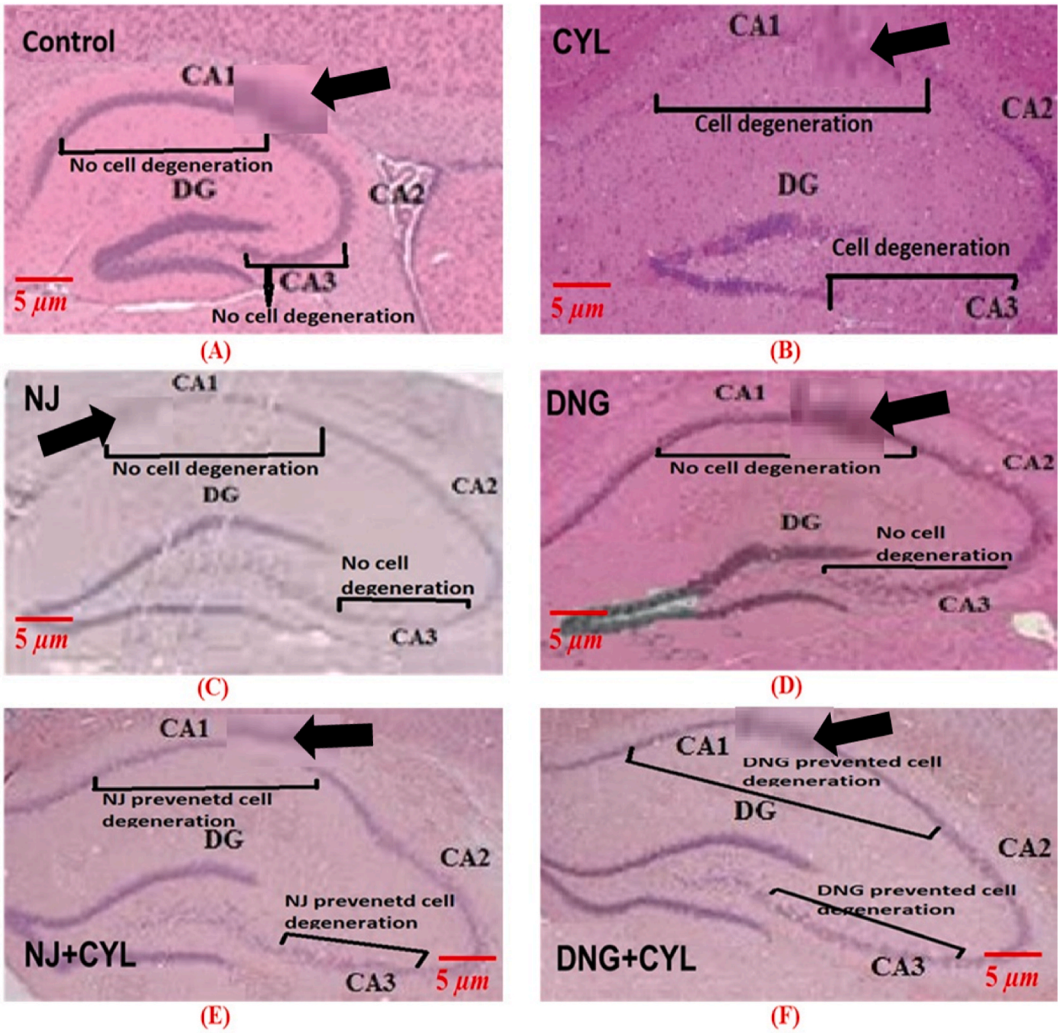

Fig. 8. Histopathological presentation of NJ and DNG in the hippocampus of CYL-challenged mice. Morphological changes were visualized under a phase contrast microscope. The mouse hippocampus was stained with hematoxylin and eosin (20×). [A]: Normal-vehicle treated [B]: Section treated with CYL. [C and D]: Sections treated with NJ and DNG respectively. [E and F]: Sections treated with NJ + CYL and DNG + CYL, respectively.

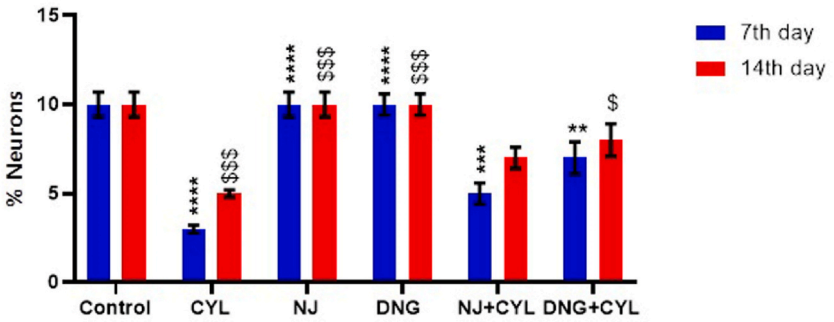

Fig. 9. Quantitative histological assessment of NJ and DNG in the hippocampus of CYL-challenged mice. All the data are presented as the mean ± SEM (n = 6) and data were analyzed by two-way ANOVA followed by Tukey's multiple comparisons test. The obtained p values were: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; $p < 0.05, $$p < 0.01, $$$p < 0.001.

groups in flavonoids mediate their antioxidant effects by scavenging free radicals [60]. Therefore, the results of the present study clearly demonstrate the potential antioxidant activity and free radical scavenging abilities of DNG.

Naringenin was found to reduce AChE activity and enhance the antioxidant defense system, which includes GSH, SOD and CAT by lowering hippocampal MDA, an indicator of lipid peroxidation [61]. The hopeful benefits of memory recovery were attributed by researchers to naringenin's AChE inhibitory action [62]. Rahigude et al. reported that after 58 days of chronic administration of naringenin (50 mg/kg bwt/day, p.o.), rats with type 2 diabetes exhibited significant improvements in MI, oxidative stress, and AChE activity [63]. These results point to naringenin as a potentially useful and multitarget neuroprotective substance. Overall, the results showed that naringenin has therapeutic potential by overcoming cognitive impairment by countering oxidative stress and neuroinflammation.

As a result, (−)epicatechin (EC), a pure flavanol of particular significance, crosses the blood–brain barrier (BBB) and may have an immediate impact on brain function. Here, we demonstrated the presence of (−)epicatechin in noni and its protective effect against CYL-induced short-term MI in mice. This effect is further strengthened when combined with (−)epicatechin, which increases hippocampal activity and memory function. Our result is an agreement with van Praag et al., stated that epicatechins are plant-derived flavanols make significant vasculature increases in the dentate gyrus. Reported that *M. citrifolia* fruit extracts contain high levels of catechins at 53.7 ± 5.7 mg/g. In their study described that administration noni fruit juice might recover the stress-induced impairment of blood vessel density in the hippocampus of mice which leads to improve memory by means of catechins contained in noni juice [64].

Hend et al., stated that naringenin improves behavioral, neurochemical, immunohistochemical, and molecular parameters in the rat cerebellum of AlCl3-induced Alzheimer's disease (AD); these protective effects may be highly ascribed to its antioxidant and autophagic regulatory properties of naringenin. suggested that naringenin could contribute to the combat of oxidative and autophagic stress in the cerebellum of AlCl3-induced AD. It's clearly indicates that naringenin compound can protect the stress induced neurodegenerative disease including memory [65].

In the present study, all of these results point to the long-lasting anatomical and functional alterations in the mouse brain caused by (−)epicatechin and naringenin present in divine noni. To clarify the significance of these concerns in MI, more research on their impacts is needed. Due to the presence of a high extent of these phytochemicals in noni fruit, it is documented as an antioxidant and anti-inflammatory agent due to the presence of Naringenin, (−)epicatechin, (+)-Catechin, Glucogallin, Ursolic acid etc. [66–70].

The existence of CYL-induced MI is usually accepted, but several details of this process remain unclear [71]. The impacted cognitive areas and the periods of cognitive loss are not consistent, according to previous research [72–74]. As stated by earlier in-vivo research, a single dose of 40 mg/kg CYL produces chemobrain, which has been found in a step-down test. However, the impairment in the step-down inhibitory avoidance test improved after 1 week of CYL treatment. This finding suggested that a CYL injection of 40 mg/kg is sufficient to interrupt cognitive function in mice, which may recover after 1 week [75]. A similar result was obtained in our study, where 40 mg/kg b.w. of CYL induced hippocampus-dependent MI on the 7th day, which was recovered and observed on the 14th day in the ELT and TSTQ in the MWM (Figs. 4 and 5). This finding implies that a single dose of 40 mg/kg CYL can briefly cause MI. Noni treatment had a preventive effect on CYL-induced MI in the present study.

Findings from previous research suggest that CYL may have an immediate effect on cells dividing in the subgranular zone of the adult dentate gyrus and that during this time, animals may become cognitively impaired. However, it appears likely that the decline in cell division and spatial cognition is mild and reversible. Aldehyde dehydrogenase-3 is an enzyme found in the brain that breaks down CYL into non-toxic metabolites [75]. This may be because the effects of CYL may be less long lasting than those of other chemotherapeutic drugs.

Our study is the first to show that DNG has a defensive effect on cyclophosphamide-induced short-term memory deficits in Swiss albino mice. Furthermore, the literature suggests another possible mechanism by which CYL inhibits hippocampal neurogenesis. The metabolites of CYL, i.e., acrolein or phosphoramide mustard, cause side effects [76]. This decreases cellular resistance to oxidative stress and leads to BBB damage, hence permitting the entry of possible neurotoxic substances, i.e., cytokines, into the brain and causing cell death in the CA1 and CA3 regions of the hippocampus, which are responsible for memory; this possible toxicity of CYL may be due to the accumulation of ROS in the brain [77]. In brain tissue antioxidant estimation, we found that CYL reduced the levels of the neuronal antioxidants i.e. GSH, SOD, CAT, and GST and simultaneously augmented the MDA and AChE levels. An earlier study demonstrated that antioxidants are closely associated with the direct removal of ROS. Hence, the depletion of these antioxidant molecules may result in many detrimental impacts owing to the accumulation of superoxide radicals and hydrogen peroxide, which are interrelated with neurodegenerative disorders, including MI [78–80].

Supplementing NJ and DNG for 14 consecutive days improved the concentrations of these antioxidant enzymes, resulting in defense against free radicals. Taken together, these findings indicate that oral consumption of noni in healthy mice reduced oxidative damage to the brain in response to CYL-induced oxidative stress and augmented the concentrations of antioxidant enzymes (Fig. 6) while diminishing the levels of MDA and AChE in response to CYL (Fig. 7). These impacts of the antioxidant properties of the noni may be related to improved performance in the MWM behavioral test. A histopathological study revealed that CYL treatment-induced degeneration of cells in the CA1 and CA3 regions of the hippocampus may be due to the depletion of antioxidant enzymes, which confirmed that CYL treatment induces MI and that this effect is protected by noni supplementation. The quantitative analysis of histopathology confirmed the degenerated cells by CYL treatment and significantly improved by noni (Fig. 9).

5. Conclusions

The present study clearly demonstrated the potential antioxidant activity of the divine noni, which may be caused by the wide variety of functional compounds in the plant, as shown by the FTIR and LC-MS spectral analysis. These results suggest that CYL may

cause MI to a short extent. The pattern of neurogenesis following CYL treatment is consistent with the dependence of the hippocampus on MI, indicating that the extent of MI may be related to cholinergic dysfunction and diminished levels of antioxidant enzymes in the brain. Thus, the neuroprotective activity of divine noni may or may not be directly related to its antioxidant activity. Further molecular studies are required to clarify whether CYL induces short-term MI in a mouse model.

Availability of data and materials

All the data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

The study was approved by the Institutional Animal Ethical Committee (IAEC No. 162/2016) of JSS College of Pharmacy, JSS University, Mysuru, India.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Mohammad Ali: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Shahid Ud Din Wani:** Writing – review & editing, Visualization, Validation, Software, Resources, Investigation, Formal analysis, Data curation. **S.N. Manjula:** Supervision, Project administration, Conceptualization. **K. Mruthunjaya:** Project administration. **Faiyaz Shakeel:** Writing – review & editing, Validation, Project administration, Investigation, Funding acquisition, Data curation. **Bharathi DR:** Resources. **Sathvik B. Sridhar:** Resources. **Ishfaq Mohiuddin:** Software. **Reyaz Hassan Mir:** Validation, Data curation. **Tathagata Dey:** Software.

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.

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References

[1] E.T. Olayinka, A. Ore, O.S. Ola, O.A. Adeyemo, Ameliorative effect of gallic acid on cyclophosphamide-induced oxida-tive injury and hepatic dysfunction in rats, Med. Sci. 3 (2015) 78–92.
[2] S.S. Roy, P. Chakraborty, S. Bhattacharya, Intervention in cyclophosphamide induced oxidative stress and DNA dam-age by a flavonyl thiazolidinedione based organo seleno cyanate and evaluation of its efficacy during adjuvant therapy in tumor bearing mice, Eur. J. Med. Chem. 73 (2014) 195–209.
[3] S.-H. Kim, I.-C. Lee, H.-S. Baek, I.-S. Shin, C. Moon, S.-K. Bae, S.-H. Kim, J.-C. Kim, H.-C. Kim, Mechanism for the protective effect of diallyl disulfide against cyclophosphamide acute urotoxicity in rats, Food Chem. Toxicol. 64 (2014) 110–118.
[4] J. Song, L. Liu, L. Li, J. Liu, E. Song, Y. Song, Protective effects of lipoic acid and mesna on cyclophosphamide-induced haemorrhagic cystitis in mice, Cell Biochem. Funct. 32 (2014) 125–132.
[5] M. Kuhlen, K. Bleckmann, A. Moricke, M. Schrappe, S. Vieth, G. Escherich, A. Bronsema, A. Vonalt, M. Queudeville, C.M. Zwaan, et al., Neurotoxic side effects in children with refractory or relapsed T-cell malignancies treated with nelarabine based therapy, Br. J. Haematol. 179 (2019) 272–283.
[6] K.S. Senthil, S.K. Yogeeta, R. Subashini, T. Devaki, Attenuation of cyclophosphamide induced toxicity by squalene in experimental rats, Chem. Biol. Interact. 160 (2006) 252–260.
[7] Y. Mythili, P.T. Sudharsan, E. Selvakumar, P. Varalakshmi, Protective effect of DL-alpha-lipoic acid on cyclophosphamide induced oxidative cardiac injury, Chem. Biol. Interact. 151 (2004) 13–19.
[8] W. Rzeski, S. Pruskil, A. Macke, U. Felderhoff-Mueser, A.K. Reiher, F. Hoerster, C. Jansma, B. Jarosz, V. Stefovska, P. Bittigau, et al., Anticancer agents are potent neurotoxins in vitro and in vivo, Ann. Neurol. 56 (2004) 351–360.
[9] R. Nagarajan, C. Peters, P. Orchard, N. Rydholm, Report of severe neurotoxicity with cyclophosphamide, J. Pediatr. Hematol. Oncol. 22 (2000) 544–546.
[10] L. Chen, X. Xiong, X. Hou, H. Wei, J. Zhai, T. Xia, X. Gong, S. Gao, G. Feng, X. Tao, et al., Wuzhi capsule regulates chloroacetaldehyde pharmacokinetics behaviour and alleviates high-dose cyclophosphamide-induced nephrotoxicity and neurotoxicity in rats, Basic Clin. Pharmacol. Toxicol. 125 (2019) 142–151.
[11] E.J. Seo, S.M. Klauck, T. Efferth, A. Pannosian, Adaptogens in chemobrain (Part II): effect of plant extracts on chemo-therapy-induced cytotoxicity in neuroglia cells, Phytomedicine 58 (2018) E152743.
[12] Y.A. Asiri, Probucol attenuates cyclophosphamide-induced oxidative apoptosis, p53 and Bax signal expression in rat cardiac tissues, Oxid. Med. Cell. Longev. 3 (2010) 308–316.
[13] M. Nilsson, E. Perfilieva, U. Johansson, O. Orwar, P.S. Eriksson, Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory, J. Neurobiol. 39 (1999) 569–578.

[14] F.U. Fontella, I.R. Siqueira, A.P. Vasconcellos, A.S. Tabajara, C.A. Netto, C. Dalmaz, Repeated restraint stress induces oxidative damage in rat hippocampus, Neurochem. Res. 30 (2005) 105–111.
[15] K. Nagata, N. Nakashima-Kamimura, T. Mikami, I. Ohsawa, S. Ohta, Consumption of molecular hydrogen prevents the stress-induced impairments in hippocampus dependent learning tasks during chronic physical restraint in mice, Neuropsychopharmacology 34 (2008) 501–508.
[16] H.V. Praag, M.J. Lucero, G.W. Yeo, K. Stecker, N. Heivand, C. Zhao, E. Yip, M. Afanador, H. Schroeter, J. Hammer-stone, et al., Plant-derived flavanol (−) epicatechin enhances angiogenesis and retention of spatial memory in mice, J. Neurosci. 27 (2007) 5869–5878.
[17] B. Shukitt-Hale, A.N. Carey, D. Jenkins, B.M. Rabin, J.A. Joseph, Beneficial effects of fruit extracts on neuronal function and behavior in a rodent model of accelerated aging, Neurobiol. Aging 28 (2007) 1187–1194.
[18] S. Nakajima, I. Ohsawa, K. Nagata, S. Ohta, M. Ohno, T. Ijichi, T. Mikami, Oral supplementation with melon super-oxide dismutase extract promotes antioxidant defences in the brain and prevents stress-induced impairment of spatial memory, Behav. Brain Res. 200 (2009) 15–21.
[19] J. Nordberg, E.S.J. Amer, Reactive oxygen species, antioxidants and mammalian thioredoxin system, Free Radic. Biol. Med. 31 (2001) 1287–1299.
[20] B.H. Robinson, The role of manganese superoxide dismutase in health and diseases, J. Inherit. Metab. Dis. 21 (1998) 598–601.
[21] A.L. Brioukhanov, A.I. Netrusov, Catalase and superoxide dismutase: distribution, properties and physiological role in cells of strict anaerobes, Biochemistry 69 (2004) 949–962.
[22] S. Nakajima, I. Ohsawa, K. Nagata, S. Ohta, M. Ohno, T. Ijichi, et al., Oral supplementation with melon superoxide dismutase extract promotes antioxidant defences in the brain and prevents stress-induced impairment of spatial memory, Behav. Brain Res. 200 (1) (2009) 15–21.
[23] S. Umukoro, H.A. Kalejaye, B. Ben-Azu, A.M. Ajayi, Biomed. Pharmacother. 105 (2018) 714–723.
[24] S. Zhou, G. Huang, Extraction, derivatization, and antioxidant activity of *Morinda citrifolia* polysaccharide, Chem. Biol. Drug Des. 99 (2022) 603–608.
[25] K. Kamiya, Y. Tanaka, H. Endang, M. Umar, T. Satake, Chemical constituents of *Morinda citrifolia* fruits inhibit cop-per-induced low-density lipoprotein oxidation, J. Agric. Food Chem. 52 (2004) 5843–5848.
[26] K. Chandran, D.I. Shane, A. Zochedh, A.B. Sultan, K. Kathiresan, Docking simulation and ADMET prediction-based investigation on the phytochemical constituents of noni (*Morinda citrifolia*) fruit as a potential anticancer drug, Silico Pharmacol 10 (2022) E14.
[27] N. Nuengchamnong, T. Saesong, K. Ingkaninan, S. Wittaya-Areekul, Antioxidant activity and chemical constituents identification by LC-MS/MS in bio-fermented fruit drink of *Morinda citrifolia L*, Trends Sci. 20 (2023) E6498.
[28] A.C. Shovic, W.A. Whistler, Food sources of provitamin A and vitamin C in the American Pacific, Trop. Sci. 41 (2001) 199–202.
[29] G.P. de Moraes, M.V.O.B. de Alencar, L.D.S. Araujo, D.C.V. Gomes, M.F.C.J. Paz, M.T. Islam, A.A.D.C. Melo-Cavalcante, J.N. Picada, Cytogenotoxic study of aqueous fruit extract of *Morinda citrifolia* in Wistar albino rats, Orien. Pharm. Exp. Med. 19 (2019) 311–321.
[30] E.S. Almeida, D. de Oliveira, D. Hotza, Properties and applications of *Morinda citrifolia* (noni): a review, Compr. Rev. Food Sci. Food Saf. 18 (2018) 883–909.
[31] H. Sina, G. Dramane, P. Tchekounou, M.F. Assogba, K. Chabi-Sika, B. Boya, K. Socohou, A. Adjanohoun, L. Baba-Moussa, Phytochemical composition and in vitro biological activities of *Morinda citrifolia* fruit juice, Saudi J. Biol. Sci. 28 (2021) 1331–1335.
[32] M.B.D.M. Lima, A.L. dos Santos, C.A.L. Cardoso, L.C. Krause, E.B. Caramao, Studies related to the chemical composition, biological activities and toxicity of methanolic extracts of noni (*Morinda citrifolia*) fruits and leaves, Nat. Prod. Res. 36 (2022) 5868–5871.
[33] C.B. Miguel, R.V. Oliveira, W.F. Rodrigues, G. Tavares, S.C. Joinhas, M.A.G. da Cruz, C.F.D.S. Rezende, A.M.P. Alvarenga, W.D.S. Mateus, M.S. Silveira, et al., In vitro antifungal activity of *Morinda citrifolia* (noni) extract against *Candida albicans*, J. Infect. Dev. Ctries. 16 (2022) 1206–1217.
[34] M. Nagalingam, S. Rajeshkumar, S.K. Balu, M. Tharani, K. Arunachalam, Anticancer and antioxidant activity of *Morinda citrifolia* leaf mediated selenium nanoparticles, J. Nanomater. 2022 (2022) E2155772.
[35] Tharachand, I. Selvaraj, M. Avadhani, Medicinal properties of malabar tamarind [*Garcinia cambogia* (gaertn.) DESR.], Int. J. Pharmaceut. Sci. Rev. Res. 19 (2013) 101–107.
[36] M.Y. Wang, C. Su, Cancer preventive effect of *Morinda citrifolia* (Noni), Ann. N. Y. Acad. Sci. 952 (2001) 161–168.
[37] C. Jianguo, S. Xue, C. Yang, L. Hanqiao, C. Chi, H. Qiyang, Neuroprotective effects of chloroform and aqueous fractions of noni juice against t-Butyl hydroperoxide-induced oxidative damage in SH-SY5Y cells, Food Nutr. Res. 62 (2018) 1–11.
[38] Jagmohan, Organic Spectroscopy Principles and Applications, second ed., Narosa Publishing House, Daryaganj, Delhi, India, 2005.
[39] J.B. Johnson, B.E. Hoyos, J.S. Mani, M. Reynolds, J. Altvater, M. Naiker, Identification of phenolics responsible for the high antioxidant activity in Burdekin plum (Pleiogynium timoriense) fruit, Food Chem. Adv 1 (2022) 100081.
[40] A. Bouhafsoun, M.A. Yilmaz, A. Boukeloua, H. Temel, M.K. Harche, Simultaneous quantification of phenolic acids and flavonoids in Chamaerops humilis L. using LC–ESI-MS/MS, Food Sci. Technol. 38 (2018) 242–247.
[41] M. Nabi, M.I. Zargar, N. Tabassum, B.A. Ganai, S.U.D. Wani, S. Alshehri, P. Alam, F. Shakeel, Phytochemical profiling and antibacterial activity of methanol leaf extract of *Skimmia anquetilia*, Plants 11 (2022) 1667, https://doi.org/10.3390/plants11131667.
[42] I. Mohiuddin, T.R. Kumar, M.I. Zargar, S.U.D. Wani, W.A. Mahdi, S. Alshehri, P. Alam, F. Shakeel, GC-MS analysis, phytochemical screening, and antibacterial activity of *Cerana indica propolis* from kashmir region, Separations 9 (2022) 363, https://doi.org/10.3390/separations9110363.
[43] A.K. Zahi, H. Hamzah, S. Hutheyfa, M.R. Shaari, S. Sithambaram, H.H. Othman, Acute and sub-acute dermal toxicity studies of *Morinda citrifolia* L. fruit extract in Sprague Dawley rats, Asian J. Pharmaceut. Clin. Res. 8 (2015) 400–408.
[44] V.V. Charles, T.W. Michael, Morris water maze: procedures for assessing spatial and related forms of learning and memory, Nat. Protoc. 1 (2006) 848–858.
[45] A. Zeb, F. Ullah, A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods, J. Anal. Methods Chem. 2016 (2016) E9412767.
[46] M.P. Lila, F. Ali, M.R. Mohamad bagher, G. Taghi, E. Mohamadreza, Activity assay of glutathione S-transferase (GSTs) enzyme as a diagnostic biomarker for liver hydatid cyst in vitro, Iran. J. Public Health 43 (2014) 994–999.
[47] T.R. Rasheed, H.S. Mansoor, A.S. Mansoor, New colorimetric method to determine catalase mimic activity, Mater. Res. Express 7 (2020) E025405.
[48] P.S. Madon, An improved photochemical method for the rapid spectrophotometric detection of superoxide dismutase, Redox Rep. 6 (2001) 123–127.
[49] G. Gallo, G. Martino, Red blood cell glutathione peroxidase activity in female nulligravid and pregnant rats, Reprod. Biol. Endocrinol. 7 (2009) E7.
[50] P. Ni, Y. Sun, H. Dai, S. Jiang, W. Lu, Y. Wang, Z. Li, Z. Li, Colorimetric determination of the activity of acetylcholinesterase and its inhibitors by exploiting the iodide-catalyzed oxidation of 3,3′,5,5′-tetramethylbenzidine by hydrogen peroxide, Microchim. Acta 183 (2016) 2589–2595.
[51] M. Grube, O. Muter, S. Strikauska, M. Gavare, B. Limane, Application of FTIR spectroscopy for control of the medium composition during the biodegradation of nitro aromatic compounds, J. Ind. Microbiol. Biotechnol. 35 (2008) 1545–1549.
[52] L. Lyons, M. Elbeltagy, G. Bennett, P. Wigmore, The effects of cyclophosphamide on hippocampal cell proliferation and spatial working memory in rat, PLoS One 6 (2011) E21445.
[53] M. Yang, J.-S. Kim, M.-S. Song, S.-H. Kim, S.S. Kang, C.-S. Bae, J.-C. Kim, H. Wang, T. Sin, C. Moon, Cyclophosphamide impairs hippocampus-dependent learning and memory in adult mice: possible involvement of hippocampal neurogenesis in chemotherapy induced memory deficits, Neurobiol. Learn. Mem. 93 (2010) 487–494.
[54] Y. Kitamura, S. Hattori, S. Yoneda, S. Watanabe, E. Kanemoto, M. Sugimoto, T. Kawai, A. Machida, H. Kanzaki, I. Miyazaki, et al., Doxorubicin and cyclophosphamide treatment produces anxiety-like behaviour and spatial cognition impairment in rats: possible involvement of hippocampal neurogenesis via brain-derived neurotrophic factor and cy-clin D1 regulation, Behav. Brain Res. 292 (2015) 184–193.
[55] L.A. Christie, M.M. Acharya, V.K. Parihar, A. Nguyen, V. Martirosian, C.L. Limoli, Impaired cognitive function and hippocampal neurogenesis following cancer chemotherapy, Clin. Cancer Res. 18 (2012) 1954–1965.
[56] Ali Mohammad, S.N. Manjula, Ud Din Wani Shahid, et al., Protective role of herbal formulation - divine noni against cisplatin-induced cytotoxicity in healthy cells by activating Nrf2 expression: an in-vivo and in-vitro approach, Phytomedicine 1 (2021) 1–10, 100009.
[57] T.A. Ahles, A.J. Saykin, C.T. Furstenberg, B. Cole, L.A. Mott, K. Skalla, M.B. Whedon, S. Bivens, T. Mitchell, E.R. Green-berg, et al., Neuropsychologic impact of standard-dose systemic chemotherapy in long term survivors of breast cancer and lymphoma, J. Clin. Oncol. 20 (2002) 485–493.

[58] F.S. van Dam, S.B. Schagen, M.J. Muller, W. Boogerd, E. vd Wall, M.E.D. Fortuyn, S. Rodenhuis, Impairment of cognitive function in women receiving adjuvant treatment for high-risk breast cancer: high-dose versus standard-dose chemotherapy, J. Natl. Cancer Inst. 90 (1998) 210–218.
[59] C. Jansen, C. Miaskowski, M. Dodd, G. Dowling, J. Kramer, Potential mechanisms for chemotherapy-induced impairments in cognitive function, Oncol. Nurs. Forum 32 (2005) 1151–1163.
[60] M.K. Dalia, B. Jurga, J. Valdas, M. Ramune, Chapter 21 - catechins, neuroprotection, and brain mitochondria, Mitochondrial Physiology and Vegetal Molecules (2021) 455–470.
[61] M.R. Khajevand-Khazaei, P. Ziaee, S.A. Motevalizadeh, et al., Naringenin ameliorates learning and memory impairment following systemic lipopolysaccharide challenge in the rat, Eur. J. Pharmacol. 826 (2018) 114–122, https://doi.org/10.1016/j.ejphar.2018.03.001.
[62] H.J. Heo, M.J. Kim, J.M. Lee, S.J. Choi, H.Y. Cho, B. Hong, H.K. Kim, E. Kim, D.H. Shin, Naringenin from Citrus junos has an inhibitory effect on acetylcholinesterase and a mitigating effect on amnesia, Dement. Geriatr. Cogn. Disord 17 (2004) 151–157.
[63] A. Rahigude, P. Bhutada, S. Kaulaskar, M. Aswar, K.J.N. Otari, Participation of antioxidant and cholinergic system in protective effect of naringenin against type-2 diabetes-induced memory dysfunction in rats, Neuroscience 226 (2012) 62–72.
[64] H. van Praag, M.J. Lucero, G.W. Yeo, K. Stecker, N. Heivand, C. Zhao, et al., Plant-derived flavanol (−)epicatechin enhances angiogenesis and retention of spatial memory in mice, J. Neurosci. 27 (22) (2007) 5869–5878.
[65] M.H. Hend, R.E. Mohamed, A. Eman, et al., Neuroprotective effect of naringin against cerebellar changes in Alzheimer's disease through modulation of autophagy, oxidative stress and tau expression: an experimental study, Front. Neuroanat. 16 (2022) 1–17.
[66] R. Rashmi, S. Bojan Magesh, K. Mohanram Ramkumar, S. Suryanarayanan, M. Venkata SubbaRao, Antioxidant potential of naringenin helps to protect liver tissue from streptozotocin-induced damage, Rep Biochem Mol Biol 7 (1) (2018) 76–84.
[67] U. Jug, K. Naumoska, I. Vovk, (-)-Epicatechin-An important contributor to the antioxidant activity of Japanese knotweed rhizome bark extract as determined by antioxidant activity-guided fractionation, Antioxidants 10 (1) (2021) 133, https://doi.org/10.3390/antiox10010133. Published 2021 Jan 18.
[68] I.G. Munteanu, C. Apetrei, Assessment of the antioxidant activity of catechin in nutraceuticals: comparison between a newly developed electrochemical method and spectrophotometric methods, Int. J. Mol. Sci. 23 (15) (2022) 8110, https://doi.org/10.3390/ijms23158110. Published 2022 Jul 23.
[69] A.N. Khan, R. Singh, A. Bhattacharya, et al., Glucogallin attenuates RAW 264.7 cells from arsenic trioxide induced toxicity via the NF-κB/NLRP3 pathway, Molecules 27 (16) (2022) 5263, https://doi.org/10.3390/molecules27165263. Published 2022 Aug 18.
[70] A.D. Somantri, D. Kurnia, A. Zainuddin, H.D.A. Dharsono, M.H. Satari, Action mode of ursolic acid as a natural antioxidant and inhibitor of superoxide dismutase: in vitro and in silico study, \"J. Adv. Pharm. Technol. Research\"\" (JAPTR)\" 12 (4) (2021) 389–394, https://doi.org/10.4103/japtr.japtr_90_21.
[71] V. Shilling, V. Jenkins, I.S. Trapala, The (mis)classification of chemo-fog– Methodological inconsistencies in the investigation of cognitive impairment after chemotherapy, Breast Cancer Res. Treat. 95 (2006) 125–129.
[72] T. Ahles, A. Saykin, Breast cancer chemotherapy-related cognitive dysfunction, Eur. J. Cancer Care 3 (2002) 84–90.
[73] H.G.M. Fan, N. Houédé-Tchen, Q.-L. Yi, I. Chemerynsky, F.P. Downie, K. Sabate, I.F. Tannock, Fatigue, menopausal symptoms, and cognitive function in women after adjuvant chemotherapy for breast cancer: 1- and 2-year follow-up of a prospective controlled study, J. Clin. Oncol. 23 (2005) 8025–8032.
[74] S.B. Schagen, M.J. Muller, W. Boogerd, R.M. Rosenbrand, D. van Rhijn, S. Rodenhuis, F.S.A.M. van Dam, Late effects of adjuvant chemotherapy on cognitive function: a follow-up study in breast cancer patients, Ann. Oncol. 13 (2022) 1387–1397.
[75] A.B. Reiriz, G.K. Reolon, T. Preissler, J.O. Rosado, J.A. Henriques, R. Roesler, G. Schwartsmann, Cancer chemotherapy and cognitive function in rodent models: memory impairment induced by cyclophosphamide in mice, Clin. Cancer Res. 12 (2006) 5000.
[76] O. Pekar, N. Molotski, S. Savion, A. Fein, V. Toder, A. Torchinsky, p53 regulates cyclophosphamide teratogenesis by controlling caspases 3, 8, 9 activation and NF-κB DNA binding, Reproduction 134 (2007) 379–388.
[77] E. Cecerska-Heryć, K. Krauze, A. Szczęśniak, A. Goryniak-Mikolajczyk, N. Serwin, D. Sleboda-Taront, R. Jacek, H. Heryc, A. Michalczyk, B. Dolegowska, Activity of erythrocyte antioxidant enzymes in healthy women depends on age, BMI, physical activity, and diet, J. Health Popul. Nutr. 41 (2022) E35.
[78] Y.Z. Fang, S. Yang, G. Wu, Free radicals, antioxidants, and nutrition, Nutrition 18 (2000) 872–879.
[79] S.M. Basavaraju, S. Mudhol, M.S. Peddha, S.U.D.W. Wani, K.L. Krishna, S. Mehdi, N. Kinattingal, Nanoemulsion-based piperine to enhance bioavailability for the treatment of LPS-induced depression-like behaviour in mice, Neurosci. Lett. 814 (2023) 137441, https://doi.org/10.1016/j.neulet.2023.137441.
[80] I.A. Chasoo, S.U.D.W. Wani, W.Y. Raja, Z.A. Bhat, et al., Physicochemical characterization, phytochemical analysis, and pharmacological evaluation of *Sambucus wightiana*, Arab. J. Chem. 16 (10) (2023) 105170, https://doi.org/10.1016/j.arabjc.2023.105170.