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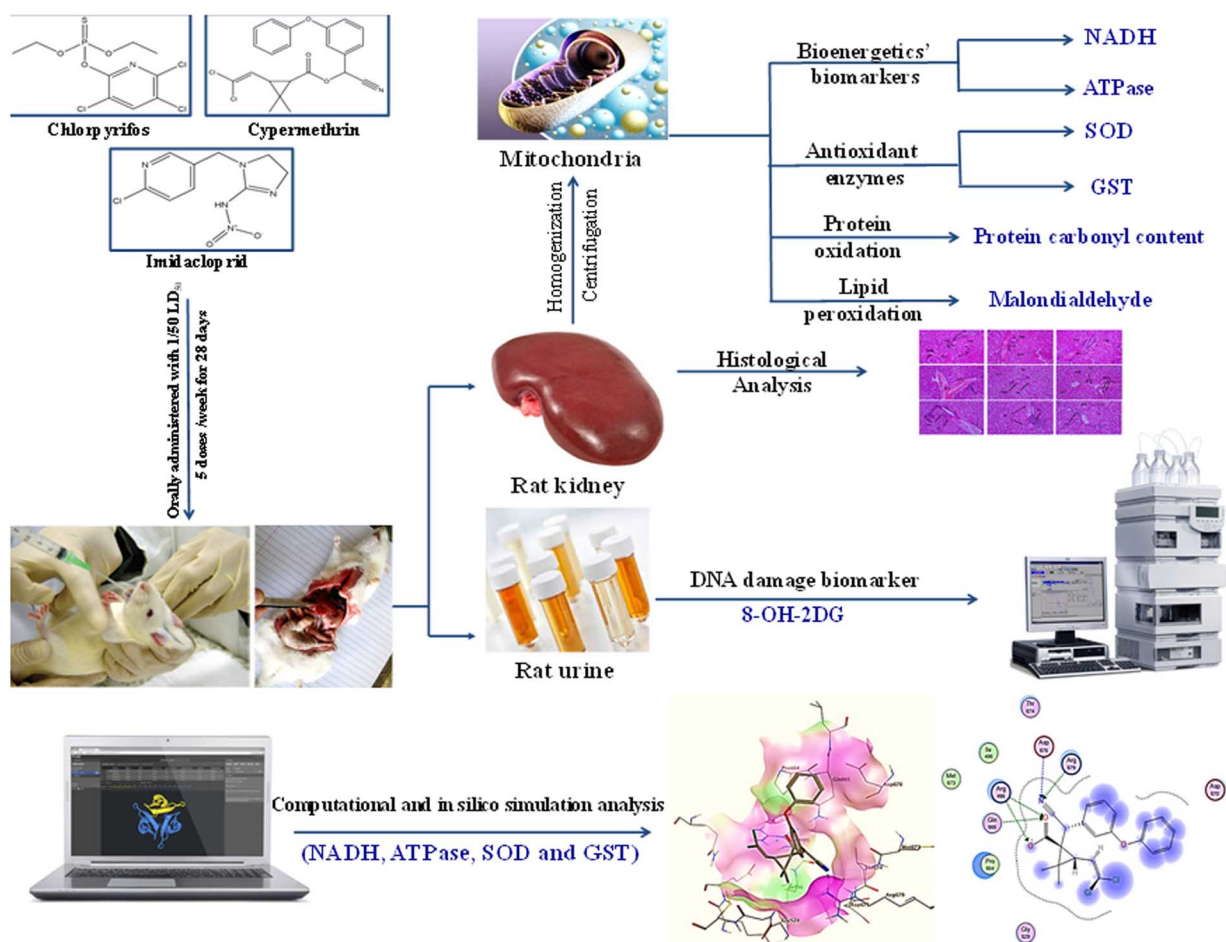
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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

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Biophysics	Biochemistry
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Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

Protective effects of Pluchea lanceolata on omeprazole induced dementia in experimental rats

Raju ASIRVATHAM[1*], Priya Prasad NEDIYARA[1], Boby JOHNS[2], Daisy Punnackal AUGUSTINE[2]

[1]Department of Pharmacology, St. Joseph's College of Pharmacy, Cherthala, Kerala, India.
[2]Department of Pharmaceutics, St. Joseph's College of Pharmacy, Cherthala, Kerala, India.

*Address for correspondence:

Dr. Raju A
Professor,
Department of Pharmacology,
St. Joseph's College of Pharmacy,Cherthala, Kerala.
E mail: rajuasirvatham@gmail.com
Phone: 9488182049

List of Abbreviations;

AD, Alzheimer's disease; CAT, Catalase; CNS, Central nervous system; CS, Conditional stimuli; EPM, Elevated plus maze; H & E, Haematoxylin and Eosin; HAEPL, Hydro alcoholic extract of Pluchea lanceolata; MDA, Malondialdehyde; ANOVA, One Way Analysis Of Variance; OS, Oxidative stress; PL, Pluchea lanceolata; PPI, Proton pump inhibitor; GSH, Reduced glutathione; SOD, Superoxide dismutase; WHO, World health organisation

Abstract

Omeprazole is the most commonly used proton pump inhibitor (PPI), a prospective cohort study reported that chronic use of PPIs causes dementia. The present study aimed to evaluate the protective effect of hydroalcoholic extract of Pluchea lanceolata (HAEPL) on omeprazole induced dementia in experimental rats. A total of 42 trained rats were divided into 7 groups, each group with six rats. The first group received normal food and water for 21 days. Second, third and fourth group animals were treated with 20 mg/kg of omeprazole for 7, 14, and 21 days respectively. Fifth, sixth and seventh groups of animals received 400 mg/kg of HAEPL + 20 mg/kg of omeprazole for 7, 14, and 21 days respectively. Behavioral studies were conducted on the 0th, 7th, 14th, and 21st days of treatment by using an actophotometer, elevated plus maze (EPM) and cook's pole climbing apparatus. On the next day of the behavior study, respective group animals were sacrificed, and the brain was isolated for estimation of antioxidants, neurotransmitters, and histopathological studies. Locomotor activity, the number of entry into open arms and time were taken to climb the poles were significantly

reduced in 20 mg/kg of omeprazole treated rats whereas activity, learning, and memory were restored in 400 mg/kg of HAEPL treated rats concerning the duration of exposure. Alteration of antioxidant enzyme, neurotransmitter level and histopathological events were found in disease control rats which were also corrected by the administration of 400 mg/kg of HAEPL. Co-administration of P.lanceolata extract diminishes the progress of dementia caused by omeprazole and may be a potential cornerstone in the treatment strategies for researchers and clinicians.

Keywords: Omeprazole; dementia; Pluchea lanceolata; antioxidants; neurotransmitters

Introduction

Among the category of proton pump inhibitors (PPIs), omeprazole is the first generation PPI and is used for the treatment of peptic ulcer, gastroesophageal reflux, and other gastric acid-related disorders. It inhibits the H+/K+-ATPase pump located in the tubular vesicular and secretory membranes of the parietal cells in the stomach thereby reducing gastric acid secretion. Data state that nearly 60–80% of patients were cured within 1–2 months of gastro-oesophageal reflux disease (GORD), hence their use is on the rise day by day (Scholten, 2007). A survey report expressed that, in the United States, PPI usage was doubled among the patient age group between 40 to 60, and moreover, 50 to 70 percent of elderly patients who are hospitalized received PPIs without any indication. Prophylactic uses of PPIs are recommended in aspirin therapy, as a gastro protectant that relieves digestive tract irritation (Makunts et al., 2019). Several studies reported that long-term usage of PPIs is associated with the development of dementia and AD (Novotny et al., 2019). Hence, therapeutic use (long term), prophylactic use, overprescribed as well as inappropriately prescribed PPIs cause adverse effects like deficiencies of vitamin B12, iron, and magnesium, osteoporotic fracture, anemia, renal damage, rhabdomyolysis, cognitive function (dementia and Alzheimer's disease (AD)). Continuous use of PPIs is also associated with a higher probability of Parkinson's Disease (PD) in the Korean population (Kim et al., 2022) Another case-control study reported that PPIs possess neuroprotective effects and decreased the risk of dementia (Hashioka et al., 2011). These controversial reports make the development of a new hypothesis to overcome the

issues such as cognitive dysfunction or impairments followed by the chronic use of PPI can be treated by a plant drug belonging to Ayurveda, traditionally used as an antiulcer and nervine tonic. One such Ayurvedic plants are "Rasna" - A controversial medicinal plant, nearly 13 plants are listed under the Rasna category in which, including *Pluchea lanceolata* (DC.) Oliv. & Hiern, (PL) (Asteraceae) is an official name of Rasna. Traditionally used for the treatment of inflammations, cough, bronchitis, piles, psoriasis, fever, uterine relaxant, nervine tonic, analgesic, laxative, ulcer and prevent the swellings of joint in arthritis (Palash et al., 2013). Hydroalcoholic extract of PL showed neuroprotective effect on stereotaxic intrahippocampal injection of endothelin-1 (ET-1)ET-1 induced focal ischemic hippocampal injury in rats model where, it reverse the pyramidal cells loss and degenerative phenotype of shrunken hyperdensed soma with pyknotic nuclei in CA1 and CA3 hippocampal neurons. The extract also exhibits antioxidant properties in ischemic rats by maintaining glutathione peroxidase and reduction of lipid peroxidation in ischemic conditions (Mundugaru et al., 2018). Similarly, hydroethanolic extract of PL showed the highest alpha-amylase and alpha-glucosidase inhibitory activity in an in vitro antidiabetic study (Sachan et al., 2019). There are not enough study to prove the neuroprotective effect on dementia by animal experimental models. So, the present study was designed to evaluate the neuroprotective effect of PL on omeprazole-induced dementia in experimental rats.

Materials and methods
Plant

The whole plant of PL was collected from Jodhpur, Rajasthan, in December 2019. Dr. J. Jameson, Plant taxonomist from the Department of Botany, St. Albert's College (Autonomous), Ernakulam, identified and authenticated, meantime herbarium specimen was also prepared and deposited (voucher specimen number is 482) at the Department of Botany, St Albert's College (Autonomous), Ernakulam, Kerala, India.

Drug

Omez (omeprazole, Dr Reddy's Laboratories Ltd, India) 20 mg capsules were procured for this study from the chemist shop at Cherthala, Kerala, India.

Extraction procedure

Roots and leaves of PL were isolated and washed with water to free them from soil particles and dried at room temperature (shade dry). Plant materials were powdered coarsely with a mechanical grinder to increase the contact between the plant materials with the solvent. The powdered materials (300g) were extracted by cold maceration using water and methanol (70:30) at room temperature for 7 days with intermittent shaking until the soluble matter has dissolved or completion of extraction. Later, the mixture was strained through muslin cloth and squeezed to remove all the remaining liquid and were passed through the Whatman filter, and then the solvent was recovered by using a rota evaporator under reduced pressure (Sarkar et al., 2012). The crude extract of PL was named hydroalcoholic extract of PL (HAEPL) and stored in a refrigerator at 4 ^{0}C in a well-tight container for further experimental purposes.

Preliminary phytochemical analysis

Tests for alkaloids, carbohydrates, glycosides, phytosterols, coumarins, flavonoids, phenolic compounds, tannins, saponins, fixed oil, protein, and amino acids were conducted as described by Mathew et al. 2021.

Determination of flavonoid content

The aluminum chloride colorimetric method has used the determination of the total flavonoid content of the sample (Asirvatham et al. 2020). For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 mL methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol (5–200 µg/mL). An amount of 0.6 mL diluted standard quercetin solutions or extracts was separately mixed with 0.6 mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 420 nm wavelength with a Double monochromator UV-2700i (Shimadzu, NA). All the determinations were carried out in triplicate.

Experimental animals

Adult rats (both sex) with body weights of 150-250g were used and were maintained under standard environmental conditions (23-25 °C, 12 hours light/12-hour dark cycle) and had free access to standard rodent pellet and water ad libitum. The animals were acclimatized in laboratory conditions for a week before the commencement of the study and were trained for the behavioral study. The method of study, treatment, and handling of animals were presented before IAEC and the committee approved the proposal number: SJCP/IAEC/2020/12/18 for the proceeding experiments on rats.

Treatment protocol

It was a 21-day study, in which a total of 42 Wistar rats (150- 250g both male and female were divided into 7 groups containing 6 animals in each. All the rats were trained in Actophotometer, Plus maze, and pole climbing apparatus before the treatment schedule to observe changes in behavior.

- **Group 1**, the animal received normal food and water for 21 days
- **Group 2,** received 20 mg/Kg of omeprazole orally for 7 days
- **Group 3**, received 20 mg/Kg of omeprazole orally for 14 days
- **Group 4,** received 20 mg/Kg of omeprazole orally for 21 days
- **Group 5,** received 20 mg/Kg of omeprazole + 400 mg/Kg of HAEPL orally for 7 days
- **Group 6,** received 20 mg/Kg of omeprazole + 400 mg/Kg of HAEPL orally for 14 days
- **Group 7,** received 20 mg/Kg of omeprazole + 400 mg/Kg of HAEPL orally for 21 days

On the 0th, 7th, 14th, and 21st day of the study, behavioral studies were conducted by using elevated plus-maze to assess memory although locomotor activity was assessed by using an actophotometer, cognitive function was assessed by using Cook's pole climbing apparatus where the response to conditioned stimuli during learning & its retention was observed. The day after the behavior study, the brain was isolated immediately after euthanasia to assess protein content as well as antioxidants enzymes malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) level. to measure neurotransmitter (acetylcholine, serotonin, dopamine, and noradrenaline) estimation and the hippocampus was separated for histopathological study.

Behavioral Studies

Trained rats were assessed for their behavioral change by using an actophotometer, elevated plus maze,

and pole climbing instruments according to the procedure of Cook and Weidley (1957) and Soman et al., (2004).

a) Actophotometer Test

Locomotor activity was assessed by using a digital actophotometer, the apparatus equipped with infrared light-sensitive photocells. Each trained animal was kept in a digital actophotometer and motor activities were observed for a period of 5min. Inside the cage, the animal movement cut off the beam of light which fell on the photocell which was counted and was recorded, values were expressed as the number of counts per 5min. Locomotor activity assessment was made in all the groups on the 0th day and after drug treatment on the 7th, 14th, and 20th day.

b) Elevated Plus Maze Test

The exteroceptive behavioral model (EPM) is widely used for evaluating learning, memory, and anxiety in rodents. It has four arms; two open arms, as well as two closed arms, are arranged opposite the central sheath which is elevated 50cm above the ground floor. Under silent and dark conditions animals were placed at the center of the apparatus. The total number of entries, as well as time spent in open and closed arms, was recorded. The elevated plus-maze tests were conducted for all the groups of trained rats on the 0th day and after drug treatment on the 7th, 14th, and 20th day, of each group of animals after drug treatment.

c) Pole Climbing Test

The cognitive function of a rat was evaluated by using Cook's Pole Climbing Apparatus where learning & memory retention was evaluated under conditional stimuli (CS). It was a wooden chamber (25 × 25 × 25cm) with stainless steel rods grid floor, 6mA shock is delivered to the floor. At the top lid, a pole (2.5cm width) was at the center of the chamber. Each rat was placed for 45seconds to explore inside the chamber. A buzzer signal followed by electric shock (unconditioned stimulus) was supplied through the steel rods grid floor for 45sec. Trained rats were learned to escape from the foot shock by pole climbing after the buzzer signal. Cut off time to climbing reaction is 10sec. The pole climbing test was conducted for all the groups of trained rats on the 0th day and after drug treatment on the 7th, 14th, and 20th day.

Estimation of antioxidants

Animals were sacrificed after 20 days of treatment by Ketamine (80 mg/Kg, i.p) + Xylazine (10 mg/Kg, i.p). The brain was removed carefully and homogenized with an ice-cold phosphate buffer of pH 7.4 to prepare brain homogenate. One part was used for the preparation of 10 %w/v homogenate in potassium chloride (0.15 M) (Homogenate I). It was centrifuged at 8000 rpm for 10 min and the supernatant thus obtained was used for (Whiteside et al., 1987 and Aebi et al., 1974) and malondialdehyde (MDA) (Ohkawa et al., 1979). The second part was used for preparation of 10% w/v homogenate in 0.25%w/v sucrose in phosphate buffer (5M, pH 7.4) (Homogenate II) and was centrifuged at 8000 rpm for 10min. The supernatant thus obtained was used for estimation of superoxide dismutase (SOD) (Kakkar et al., 1954) and glutathione (GSH) (Beutler et al., 1963). All the above antioxidant enzymes level from tissue homogenate were done by COBAS MIRA PLUS – S autoanalyser (Roche, Switzerland) using assay kits from Agappe Diagnostics, Kerala, India

Determination of total protein

In each tube, add 50μl of homogenate I, 2950μl of 0.9% NaCl, and 3000μl of Biuret reagent. Mix the contents of the tubes by shaking and warm at 37 °C for 10 min. Now cool the contents to room temperature and record the absorbance at 540 nm against blank. 1 ml of distilled water and 3 ml of Biuret reagent (Mæhre et al., 2018) in a test tube serves as the blank and the total protein was estimated by using Shimadzu UV spectrophotometer(UV-2700i, NA).

Estimation of brain neurotransmitters

In a homogenizer, brain tissue was homogenized with 5ml HCl- butanol solution for about 1min. Then it was centrifuged for 10min at 2000 rpm. Under identical conditions, 1ml of supernatant was added to a centrifuge tube and shaken with 2.5ml heptane and 0.3ml of HCl (0.1M) for 10 min. Discard the organic layer and take the aqueous phase (0.2ml) to estimate serotonin (5HT), dopamine (DA), and noradrenaline (NA). The estimation method is described by Ciarlone (1978) and Schlumpf et al., 1974, a fluorometric assay in which a fluorescent product results from the reaction with a mixture of alkaline sulfite and iodine solution (in case of DA and NA) and the reaction with ortho-phthalaldehyde solution (in case of 5-

HT). The fluorescence of DA was read at excitation 320 nm and emission 375 nm; whereas that of NA was read at excitation 380 nm and emission 480 nm. Regarding 5-HT, its fluorescence was read at excitation 355 nm and emission 470 nm using Shimadzu spectrophotofluorometer (RF-510, Japan). The results were expressed as µg/g wet tissue). This procedure was carried out at 0 °C and the estimation of acetylcholine was performed according to the procedure of Ellman (1959).

Histology of brain

Hippocampus was isolated, washed with ice-cold saline, and kept in formaldehyde (10%). Under the light microscope (Nikon, Tokyo Japan), parts were (under 10X) by a pathologist. To visualize different components of the tissue under a microscope, the sections are dyed with one or more stains. The aim of staining is to reveal cellular components; counter-stains are used to provide contrast. Hematoxylin-Eosin (H&E) staining has been used by pathologists in which, Hematoxylin stains cell nuclei blue, while Eosin stains cytoplasm and connective tissue pink (Gurcan et al., 2009).

Statistical analysis

All the in vivo study data were expressed as the Mean ± SEM of six values. The difference between treatment groups was compared to disease control by One Way Analysis Of Variance (ANOVA) followed by Newman-Keul's multiple comparison test; where, $p < 0.05$ implied significance calculation.

Results

The phytochemical screening results are shown in **Table 1.** A standard procedure was followed to test the phytochemical constituent from HAEPL. The extracts showed the presence of carbohydrates, alkaloids, glycosides, flavonoids, tannins, phytosterols, amino acids, and proteins.

Total flavonoid content present in HAEPL was estimated by using AlCl₃. The amount of flavonoids present in the extracts was expressed as mg/g (QE) and shown in **Table 2**. The flavonoid contents were estimated from the standard calibration curve of Quercetin. It was plotted on the X-axis and the corresponding absorbance was plotted on Y axis, which was represented in **Figure 1**. The concentration of total flavonoid content in the test samples was calculated from the calibration plot (y =

1.4788x + 216.9, R² = 0.9941) and expressed as mg quercetin equivalent (QE)/g of dried plant material.

Table 1 Phytochemical analysis of the HAEPL

CONSTITUENT	HAEPL
Alkaloids	+
Flavonoids	+
Tannins	+
Steroids	+
Glycosides	+
Saponins	-
Terpenoids	+
Proteins	+
Carbohydrates	+
Phenols	+

+   Colour produced (present), -   No colour produce (Absent)

Table 2 Total flavonoid content in HAEPL

Concentration µg/mL	Total flavonoid content in HAEPL mg/g (QE)
50	0.24± 0.004
100	0.45± 0.021
150	0.63± 0.021
200	0.99± 0.078
250	1.05± 0.024

Each value is expressed as Mean ± SEM (n = 3)

The effect of HAEPL on locomotor activity in omeprazole induced Neurotoxicity (Dementia) in training rats were illustrated in **Table 3.** The locomotor activity on 0th day was not significant (p>0.05) in between the trained rats in each group but on 7th, 14th, and 21st day, the locomotor activity of 400 mg/kg HAEPL and 20 mg/Kg of omeprazole treated rats showed significant (P<0.01) effect when compared with disease.

The changes in learning, memory ability, and anxiety of trained rats were recorded as the number of entries in open and closed arms in 5min were monitored for 21 days with HAEPL on omeprazole induced neurotoxicity study was illustrated in **Table 4**. On the 0th
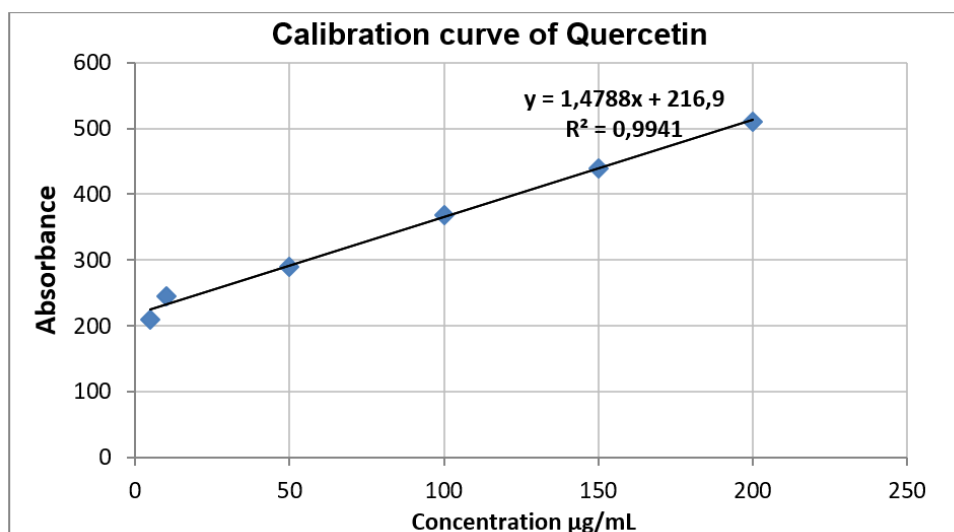
Figure 1 Calibration curve of Quercetin for determination of total flavonoid content

Table 3 Effect of HAEPL on locomotor activity of omeprazole induced Neurotoxicity (Dementia) in trained rats

Parameters of Treatment	Number of counts / 5min			
	0th Day	7th Day	14th Day	21st Day
Normal Control	206.13±0.05	220.10 ±0.04	215.17 ±0.10	225.11± 0.07
Disease Control (omeprazole 20 mg/kg)	202.46±0.03	131.61 ±0.17	108.30 ±0.05	90.505± 0.16
HAEPL (400 mg/kg+ omeprazole (20 mg/kg)	208.67±0.12	216.97±0.11^a	192.34±0.16^a	193.20±0.20^a

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where a: p<0.001.

Table 4 Effect of HAEPL on learning, memory ability and anxiety (number of entries in open & closed arm) of omeprazole induced neurotoxicity in trained rats.

Parameters of Treatment	Number of entries in open arm and closed arm/5min							
	0th Day		7th Day		14th Day		21st Day	
	Open	Closed	Open	Closed	Open	Closed	Open	Closed
Normal control	4.46 ± 0.01	29.40 ± 0.25	4.45 ± 0.05	26.91 ± 0.30	5.24 ± 0.16	24.61 ± 0.16	7.69 ± 0.08	20.10 ± 0.07
Disease control (omeprazole 20 mg/kg)	4.79 ± 0.01	19.70 ± 0.07	7.01 ± 0.04	18.08 ± 1.39	9.57 ± 0.01	17.61 ± 0.12	11.26 ± 0.01	13.91 ± 0.23
HAEP (400 mg/kg) + omeprazole (20 mg/kg)	4.24 ± 0.03	26.42 ± 0.03^a	4.31 ± 0.05 ^{a}	28.36 ± 0.09	4.73 ± 0.04 ^{a}	24.23 ± 0.04^a	5.54 ± 0.03 ^{a}	22.35 ± 0.05^a

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where a: p<0.001.

Table 5 Effect of HAEPL on conditioned avoidance response test in omeprazole induced neurotoxicity in trained rats.

Parameters of Treatment	Time taken to climb the pole (min)			
	0th Day	7th Day	14th Day	21st Day
Normal control	2.61±0.03	2.45±0.03	2.42±0.03	2.40±0.004
Disease control omeprazole (20 mg/kg)	2.49±0.05	2.73±0.002	2.93±0.001	3.14±0.001
HAEPL (400 mg/kg) + omeprazole (20 mg/kg)	2.57±0.004	2.41±0.003[a]	2.24±0.004[a]	2.40±0.004[a]

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where a: p<0.001.

Table 6 Effect of HAEPL on brain neurotransmitter level in omeprazole induced neurotoxicity in rats.

Parameters of Treatment	Acetylcholine (μmoles/minute/mg protein)	Dopamine (ngm/gm tissue)	Noradrenaline (ngm/gm tissue)	Serotonin (ngm/gm tissue)
Normal control	8.14±0.01	1.09±0.01	0.75±0.01	0.59±0.01
Disease control omeprazole (20 mg/kg)	2.01±0.03	0.75±0.01	0.23±0.06	0.26±0.09
HAEPL (400 mg/kg) + omeprazole (20 mg/kg)	7.76±0.02[a]	0.95±0.01[a]	0.69±0.01[a]	0.58±0.01[a]

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where a: p<0.001

Table 7 Effect of HAEPL on antioxidant enzymes and total protein content in the brain of omeprazole induced neurotoxicity in rats.

Treatment parameters	Days	Normal control	Disease control (omeprazole 20 mg/kg)	HAEPL (400 mg/kg) + omeprazole (20 mg/kg)
MDA (nmoles of MDA/mg wet tissue)	7	44.23±2.1	54.02±1.54	50.87±3.61
	14	46.09±5.26	68.51±2.02	45.32±1.48
	21	45.68 ± 0.05	78.62 ± 0.04	44.59 ± 0.08[a]
GSH (Millimole/g)	7	57.17±0.69	34.72±1.38	44.32±1.73
	14	56.28±1.92	21.49±2.80	41.52±1.53
	21	55.80 ± 0.05	17.76 ± 0.07	39.36 ± 0.03[a]
CAT (U/Mol)	7	33.47±0.79	27.51±0.58	31.62±0.72
	14	35.09±1.84	19.60±1.01	27.49±1.51
	21	35.22 ± 0.04	11.44 ± 0.04	22.46 ± 0.02[a]
SOD (U/g tissue)	7	74.82±1.42	54.50±2.36	66.53±1.48
	14	72.97±1.61	41.62±0.94	60.71±1.59
	21	73.77 ± 0.06	33.42 ± 0.04	54.75 ± 0.08[a]
Total protein content (mg/g)	7	39.01±2.42	29.72±1.05	38.56±0.61
	14	38.48±1.41	27.63±1.84	33.48±1.69
	21	38.85±0.15	25.02±0.16	34.60±0.07[a]

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where a: p<0.001.

day, the number of entries in open arms and closed arms were not much changed between the trained rats in all groups but the number of the entry in open arms periodically changed from 4.79 to 11.26 and in closed arms changed from 19.70 to 13.91 after 21 days administration of 20 mg/Kg of omeprazole whereas rats which were treated simultaneously with 400 mg/Kg of HAEPL, significantly (p<0.001) restored the open and closes arms entries nearby normal rats.

In the conditioned avoidance response test, the time taken to climb the pole was monitored in omeprazole and HAEPL treated rats were illustrated in **Table 5**. On the 0th day, the time taken to climb the pole was not much changed among the trained rats in all groups but after the administration of 20 mg/Kg of omeprazole once a day for 21 days gradual increase time from 2.49 to 3.14 whereas rats which were treated simultaneously with 400 mg/Kg of HAEPL, significantly (p<0.001) restored the memory closed to normal rats.

Table 6 showed the effect of HAEPL on brain neurotransmitter levels in omeprazole-induced neurotoxicity in rats. Acetylcholine, dopamine, noradrenaline, and serotonin levels were decreased in the disease control group and it was more significantly (p<0.001) increased in rats that were treated with 400 mg/kg of HAEPL.

The effect of HAEPL on antioxidant enzymes and protein content in the brain of omeprazole-induced neurotoxicity in rats was shown in **Table 7**. The protein content, SOD, CAT, and GSH levels were decreased in omeprazole alone treated animals at the dose of 20 mg/kg but MDA level was higher in the same treatment animals. Upon 21days, 400 mg/kg of HAEPL treatment made significant (p<0.001) restoration of protein and other antioxidant enzymes as like that of normal rats.

Table 8 showed the histopathological reports of the hippocampus of the brain of omeprazole-induced dementia with different duration of HAEPL treatment groups (n=6). The hippocampus section of the disease control rat showed mild to moderate congestion in the cerebral as well as meningeal parts with diffused area whereas 21 days of treatment with HAEPL made that all the cellular units were within normal functional limits.
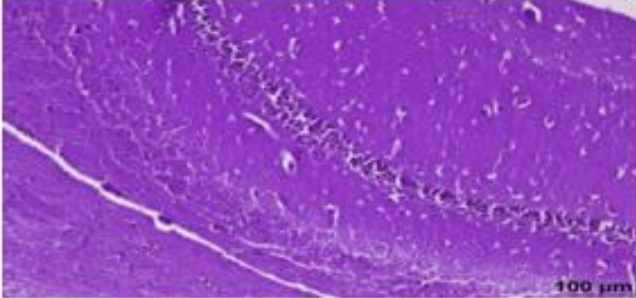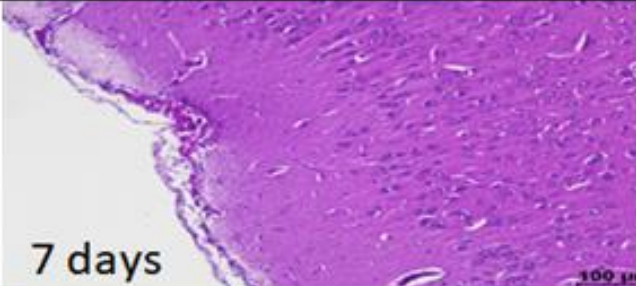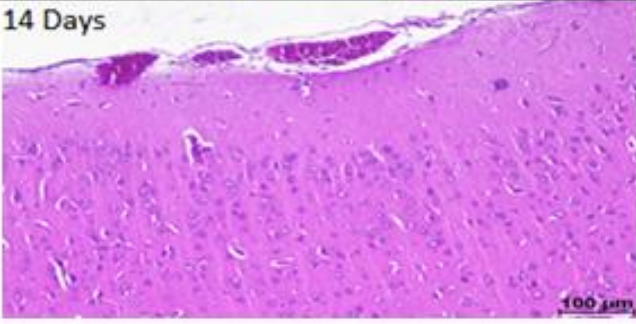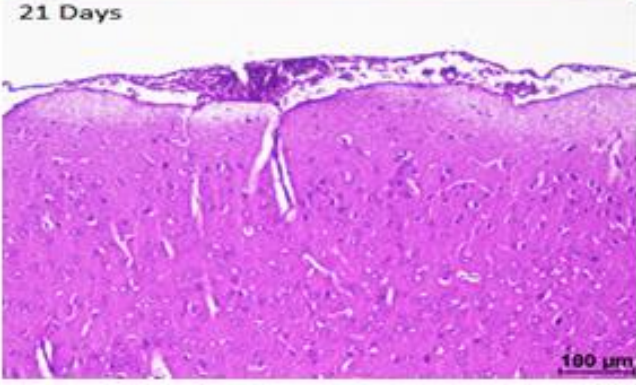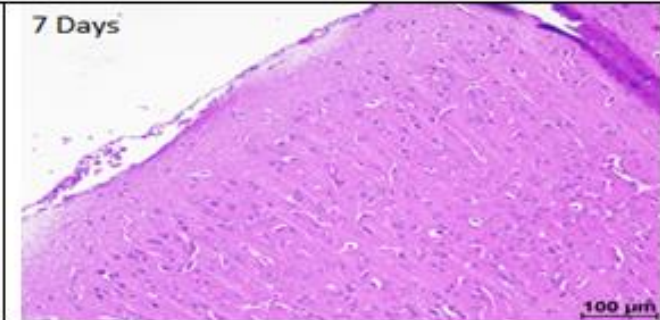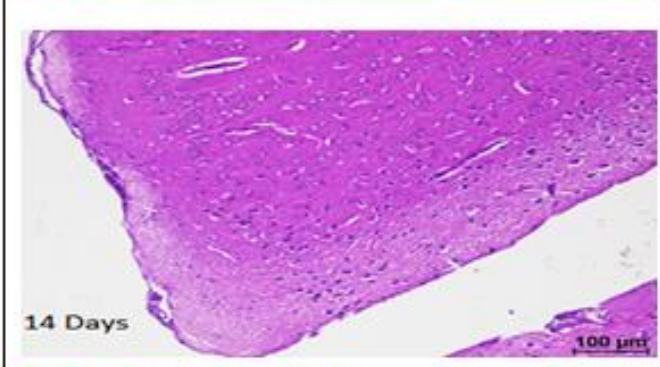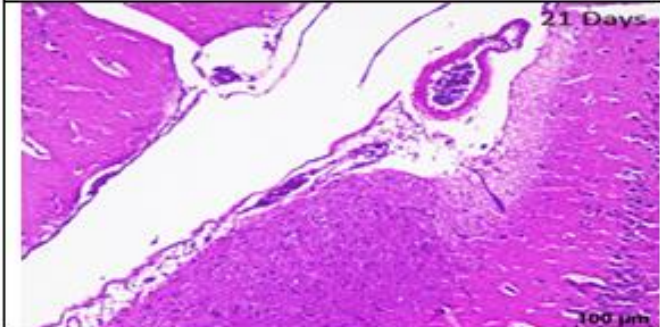
Discussion

Naturally occurring phytochemical constituents in the plants show biologically significance by playing an essential role in the plants defending themselves against various pathogenic microbes as well as reducing the risk of different kinds of disease with their antioxidant and anti-inflammatory potential. The present study results revealed the presence of phytochemicals, alkaloids, flavonoids, glycosides, terpenoids, tannins, phenols, and steroids. These phytoconstituents help to search for various bioactive agents which can be used in the synthesis of useful drugs. Phenolic and flavonoid compounds have a lot of health-benefiting properties and are considered the most important classes of phytochemicals (Pant et al., 2017). Flavonoids, including flavones, flavonols, and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plants rich in flavonoids and phenolic components have been reported on their effective antioxidants, anticancer, antibacterial, cardioprotective agents, anti-inflammation, immune system promotion, skin protection from UV radiation, and interesting candidate for pharmaceutical and medical application. Hence, recent studies and research are focusing on flavonoids and the other phenolic compounds from a medicinal plant because of their versatile benefits for human health (Tungmunnithum et al., 2018). The present study also estimated the total flavonoid content which is commonly known as the largest phytochemical molecule with antioxidant properties from plants.

PPIs, suppress gastric acid secretion and are indicated for the treatment of various gastrointestinal problems like chronic ulcers, gastroesophageal reflux, Zollinger Ellison syndrome, and digestive bleeding problems (Kumar et al., 2020). More than 50% of received this medication, not for a proper indication, and it was consumed more by geriatric patients. Nowadays, it is an add-on drug in most prescriptions to overcome the adverse effect of other prescribed medications. It could have deleterious values on the health system either used by the geriatric patient based on prescription or certain self-medication events. There is the risk of development of dementia among elder patients was reported by three epidemiological studies. In the cognitive declining process, motor impairments are the first event which are the well-known biomarkers that help to predict dementia syndromes. It may due to in malabsorption of vitamin B12 (Cooksey et al., 2020). The incident of dementia is marked by the comparison of motor function tests between the poor performances vs better performance where there is an

Table 8 Histopathology report of rat hippocampus

Group & Treatment	Photomicrograph	Report
Normal		Section showed that all the cellular functional units were within normal limits
Disease Control (omeprazole 20 mg/Kg)	7 days	Section showed mild Congestion in the cerebral/meningeal part and mild diffused area
	14 Days	Section showed mild Congestion in the cerebral/meningeal part and mild diffused area.
	21 Days	Section showed moderate Congestion in cerebral as well as meningeal part and mild diffused area

The above table showed the histopathological reports of the hippocampus of the brain of omeprazole induced dementia with different duration of HAEPL treatment groups (n=6).

increased risk of the incident found with poor performances category (Kueper et al. 2017). In the present study also the decline of locomotor activity was found with the duration of treatment but treatment with HAEPL restored the locomotor activity.

According to WHO, the last of dementia is memory disturbances, which are serious physical signs and symptoms that become more obvious (https://www.who.int, 2021). Prolonged feelings of apparent stress and anxiety cause damage to selective neurons which wires the stress-associated behavior thereby subsidizing cognitive impairment followed by declining working memory, speed, and retaining information (Wilson et al. 2011). EPM was employed for the evaluation of learning and memory in which the number of entries in both open and closed arms as well as the time is taken to move from open arm to closed arm or vice versa were calculated as transfer latency (Jafarian et al., 2019). Meantime the mice can distinguish between the open arm and the closed arm and recollect the location of both arms (Ltoh et al., 1990). If the animals showed a decrease in transfer latency period or percentage of entry to the open arms during the second trial as well as consecutive experimental periods indicated that improvement of memory and learning. This reduction was reversed by continuous administration of 20 mg/Kg of omeprazole for 21 days indicating that the given drug affects memory and learning. However, 400 mg/Kg of HAEPL produced a better memory-enhancing effect in mice.

From Cooks pole climbing apparatus, memory retrieval capacity was determined as the ability of an animal to retention the acquire memory process. It was indicated by increasing number of avoidance response. Researchers reported that the time taken to climb the pole

was increased in the animals exposed to the neurotoxic drugs and was due to dementia (Salwa et al., 2022). In the present study, time taken to climb the pole was noted where the time taken to escape from the electric shock field was reduced as that of normal trained rats after continuous 20 days treatment of HAEPL.

Development of memory in the brain has certain repeated phrases like acquisition, association, recovery, and extinction phases in which learning of different behavior belongs to the acquisition phase, maintenance or storage of earned memory belong to another phase, and so on with respective phase function. A continuous acquisition phase is required for learning novel information and retaining the same moreover, the functional cholinergic pathway is essential for memory (Gallagher, 1997). Acetylcholine is essential in learning and memory In dementia, markedly reduced concentrations of acetylcholine in the hippocampus and neocortex, caused by degeneration of cholinergic neurons. Individuals with AD have low levels of ACh. Some research suggests that plaques may be one of the reasons for low levels of ACh because they increase the activity of a chemical called acetylcholinesterase, which is involved in breaking down Ach, resulting in lower levels of acetylcholine in the brain. Researchers also found that, AD damages cells that produce and use acetylcholine (Francis, 2005) and this type of impairment in cholinergic system leads to loss of memory thereby the time taken to climb the pole was increased with only omeprazole treated rats and was restored in 400 mg/Kg of HAEPL treated rats.

Dopamine also plays an important role in motor functions. The mitochondrial depletion followed by energy dysfunction affects the dopaminergic neurons because it requires more energy for effective function. The severity of neurotoxicity (dementia) is associated with the degeneration of dopaminergic neurons in the striatum, cerebellum and the mid brain resulting in reduction of brain dopamine content. Moreover, certain plant nutrients serve as main precursors in the synthesis of neurotransmitters such as epinephrine, acetylcholine, serotonin, and dopamine (Johnson et al., 2020). Treatment with 400 mg/Kg of HAEPL increases the level of dopamine.

There have been conflicting reports on levels of noradrenaline in the brain; with some studies showing a decrease in noradrenaline while others showing that noradrenaline levels in AD patients remain constant, or even elevated. One possible theory is that in the early stages of locus coeruleus neuron loss, the remaining neurons undergo compensatory mechanisms to maintain the noradrenaline level. However, as the disease progresses and more neurons are lost, it may become impossible for the remaining neurons to totally compensate. It is also possible that the brain over-compensates for the neuronal loss, which can account for the increased noradrenaline levels reported in the later stages of the disease. Eventually, however, if the disease progresses enough, the loss of locus coeruleus neurons will be too great to overcome and noradrenaline levels will decline. Few research reports say that decreases in noradrenaline concentration in various brain regions also found in patients with Dementia, which reflect the level of cognitive deficit. Moreover, higher concentrations of the metabolite noradrenaline were also found in patient with Dementia. This increased metabolism of noradrenaline is a possible cause of decreased noradrenaline levels (Gannon et al., 2015). However, the present study animals which were treated with 400 mg/Kg of HAEPL showed increased the level of noradrenaline in the brain.

Various animal and clinical studies reports have indicated the role of 5-HT and its receptors in different aspects of cognitive dysfunction. Tandospirone is a 5-HT1A partial agonist which showed a dose-dependent decline in explicit verbal memory in healthy volunteers. Indeed, increased 5-HT1A receptor density correlated with cognitive impairment observed in AD and provided the basis for the use of 5-HT1A antagonists in AD treatment (Hala et al., 2014). It has generally been presumed that neuronal death contributes to the loss of neurotransmitters, but instead depletion of neurotransmitters also contributes to neurodegeneration (Alisky, 2006; Nakamura et al., 1984). The current study also reduced neurotransmitters found with only omeprazole treated rats and was restored in 400 mg/Kg of HAEPL treated rats.

In the pathogenesis of dementia, oxidative stress (OS) plays a major role in all types of CNS disorders. Researchers also reported that lipid peroxidation was high in AD, where the elevated MDA was considered a marker for the level of OS. It also causes memory impairment in rats which is associated with elevated brain lipid peroxides and reduced brain stores of antioxidants (Hala et al., 2014). This was apparent in the present experiments by increased brain lipid peroxidation (MDA level) parallel to reduced GSH stores by 20 mg/ Kg of omeperazole. Treatment with 400 mg/Kg of HAEPL inhibited the lipid peroxidation

significantly and decreased MDA level and raised the GSH levels.

While oxidative metabolism, free radicals are generated and are neutralized by the cellular antioxidant system where SOD catalyzes the formation of hydrogen peroxide from superoxide radicals, toxic hydroxyl radicals are removed by CAT and GSH. OS can reduce the activity of antioxidant enzymes (SOD, CAT, and GSH), together with an increase in lipid peroxidation which leads to a high level of MDA because brain cells are more sensitive to oxygen free radicals, which could be due to either a decrease in free radical defenses (or an increase in free radical formation, or both. They also reported that lipid peroxidation was high in the AD tissue resulting in raised MDA levels and a statistically significant decrease in SOD, GSH, and CAT (Marcus et al. 1998; Shichiri, 2014). The present study also found the same results as the increased level of MDA and reduction of SOD, CAT, and GSH with only omeprazole treated rats and was restored in 400 mg/Kg of HAEPL treated rats.

Alterations in the hippocampal formation are also likely to cause cognitive deficits in certain animal models. Recent studies have revealed considerable structural integrity in the hippocampus, even in aged rats with the most impaired spatial memory (Gallagher, 1997). Histopathological hippocampus reports reveal the same in animals treated with only omeprazole treated rats and were within the normal limit is 400 mg/Kg of HAEPL treated rats.

In conclusion, long-term use of PPIs could increase the risk of developing dementia and was demonstrated in this animal study result of change in behavior, antioxidant enzyme system, neurotransmitter level, and photomicroscope of the hippocampus region of brain cell. Herbs like PL and herbal products are beneficial to the patient who takes PPIs for therapeutic as well as prophylactic use for a long time which diminishes the progress of dementia. The intervention of phytotherapy or tailoring therapy, which involves the use of allopathy drugs with Ayurvedic medicine, may be a potential cornerstone in the treatment strategies. It is palpable that there has been expanding need for such therapeutic intervention for researchers and clinicians.

Declaration

The research protocol was approved by the institutional animal ethical committee (IAEC) of St. Joseph's College of Pharmacy, Cherthala (Proposal number: SJCP/IAEC/2020/12/18)

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PPN performed the all experiments; RA wrote the manuscript; DPA analyzed all data; and BJG edited the manuscript. All authors read and approved the final manuscript.

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