INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Benincasa Hispida Reversed D-galactose-induced oxidative Stress and neurodegeneration-mediated Cognitive Impairment in Aged Rats

Pooja R. C.[1], Dr. Bharathi D. R.*

Ph.D. Research scholar, Department of Pharmacology, Sri Adichunchanagiri College of Pharmacy, B.G. Nagara-571448, Karnataka, India

Dean of pharmacy & Professor, Department of Pharmacology, Sri Adichunchanagiri college of pharmacy, B.G. Nagara-571448, Karnataka, India

ARTICLE INFO

Received: 12 Oct 2023
Accepted: 13 Oct 2023
Published: 17 Oct 2023

Keywords:
Benincasa hispida (BH), Aging, Cognitive impairment, Neuroinflammatory, Oxidative stress, Western blotting

DOI:
10.5281/zenodo.10014344

ABSTRACT

Age-related Cognitive impairment is a common issue that is frequently brought on by oxidative stress and neurodegeneration in the brain. **Material & methods:** *Benincasa hispida*, commonly known as wax gourd, is widely used as a vegetable with various medicinal properties. it belongs to the family of Cucurbitaceae. The study evaluates the cognitive role of a hydro-ethanolic seed extract of *Benincasa hispida* (BH) against D-gal-induced in aged rats. Old male rats were divided into six groups: The control group received normal saline, donepezil (2 mg/kg b.w.t., i.p.); D-gal (300 mg/kg b.w.t., i.p.); D-gal +BH (150 mg/kg b.w.t., o.p.); D-gal BH (250 mg/kg b.w.t., o.p.); and D-gal BH (300 mg/kg b.wt. **Results and Discussion:** The behavioral study depicted that D-gal significantly alters locomotor activity. Biochemical studies showed that D-gal significantly increases oxidative stress in rat brains. Histopathological study showed that D-gal disturbs the normal architecture of hippocampal and cortical cells, indicating degeneration in these brain areas. D-gal and BH co-treatment for 42 days attenuated the behavioural, biochemical, and neuroanatomic damages caused by D-gal; it markedly suppressed the D-gal-induced oxidative stress. **Conclusion:** Thus, this study shows that BH can protect the brain from the adverse effects of D-gal (e.g., memory loss and cognitive impairment) by modulating oxidative stress.

INTRODUCTION

Aging process and neurodegenerative glitches are both thought to be knowingly mediated by Cognitive impairment, and oxidative stress [1]. Oxidative stress and neuroinflammation have been linked to different neurodegenerative illnesses [2]. The number of older people aged 60 or over will grow from 962 million to nearly 2.1 billion by

***Corresponding Author:** Dr. Bharathi D. R.
Address: *Dean of pharmacy & Professor, Department of Pharmacology, Sri Adichunchanagiri college of pharmacy, B.G. Nagara-571448, Karnataka, India*
Email : rambha.eesh@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2050 [3]. D-galactose is a monosaccharide normally converted into glucose by galactose-1-phosphate uridylyl transferase and galactokinase [4]. Long-term d-gal treatment can result in an enzymatic excess. Also, in high concentrations, scientists believe that d-gal may react with long-lived proteins to create amino-fructose at an early stage, resulting in irreversible protein cross-linking to advanced glycation end products (AGEs) [5-6]. The underlying mechanisms of apoptosis control, it is essential to understand the complicated interactions between the Bax and Bcl-2 proteins. Understanding the signaling pathways that activate and interact with these proteins may be helpful in developing specific drugs that might encourage apoptosis in increase cell survival in neurological diseases [7]. Donepezil is a reactive metabolite that is metabolized by the CYP 450 isoenzymes 2D6 and 3A4 and undergoes glucuronidation with a well-known mechanism [8]. In recent years, natural compounds with potential aging/cognitive properties have gained attention in the scientific community BH, commonly known as wax gourd is a widely consumed vegetable with various medicinal properties. It belongs to the Cucurbitaceae family. Various wax gourd parts such as seed peel, core, and pulp show different levels of antioxidation capacity, and fresh seeds appeared to have the highest antioxidant activity. According to the Sanskrit texts, it is useful in insanity, epilepsy, nervous diseases, dyspepsia, etc. Some scientific studies have been carried out to reveal anti-inflammatory, antioxidant, and anti-convulsant. The major constituents of these seeds are triterpenoids, flavonoids, glycosides, saccharides, proteins, carotenes, vitamins, minerals, ß-sitosterol, and uronic acid [9]. This study was aimed at investigating the hydro-ethanolic seed extract that is readily from fresh fruit seeds and performed antioxidant activities and western-blotting assay with apoptosis markers, which have a main role in aging. The latter effect is the first such study on the fruit seed. The study conclusions will contribute to the expanding figure of knowledge on natural compounds as therapeutic interventions for age-related cognitive decline.

2. MATERIAL AND METHODS

2.1 MATERIAL

2.2 Chemical and reagents

D-galactose, Sodium dodecyl sulfate, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Nitric oxide, Hydrogen peroxide, Acetylcholinesterase, *Glutathione,* Advanced oxidation protein products, Malondialdehyde, and Glutathione S-Transferase enzyme all chemicals are purchased from Sigma-*Aldrich*.

2.3 Collection of plant specimen

Benincasa hispida fruit was collected in an area of the Nelamangala market in the month of May. The specimen was authenticated by a Pharmacognosiste professor Dr. Nandeesh, Sree Siddaganga College of Pharmacy, B.H. Road, Tumkur. specimen number SSCP/No/15/22.

3. Preparation of plant specimen

The fresh 200g seed of *Benincasa hispida* was ground using a mixer. The collected homogenate paste was mixed with (70:20 v/v) Hydroethanolic solvent for 72 hours by cold maceration process. The homogenate was filtered via muslin cloth followed by Whatman No. 1 filter paper and lyophilized to obtain a dry powder of seed extract. The lyophilized powder extract will be stored in air-tight amber-color bottles, The Yield percentage (w/w) from the dried extracts was calculated [10].

4. METHODS

4.1 Animal housing

Thirty-six-week-old Wistar male rats 180- 200 g were used. Animals were maintained under standard laboratory conditions temperature of 25±2°C, relative humidity of 55±5%, and a 12 h/12 h light/dark cycle. Standard vivarium conditions with free access to food and water *ad libitum* all the procedures are done according to

OECD 423 guidelines [11]. Provided proper care and handling according to the institutional animal ethics committee of the National Research Centre SACCP-IAEC/2022-02/69. This experiment was performed in Sri Adichunchanagiri College of Pharmacy, BG. Nagar-571448, Karnataka, India.

4.2 Dose

The dose range was selected from previous acute-chronic toxicity studies [12].

5 EXPERIMENT

Thirty six animals were divided into six groups, 10-12 weeks Male Wistar rats weighing 230-260 g

Group 1 – a control group (n=6);

Group 2 – a group with Donepezil 2 mg/kg·b.wt. (n=6);

Group 3 – a group with D-galactose 300 mg/kg·b.wt. (n=6);

Group 4 – a group with BH 150 mg/kg·b.wt. + D-galactose 300 mg/kg·b.wt. (n=6);

Group 5 – a group with BH 250 mg/kg·b.wt. + D-galactose 300 mg/kg·b.wt. (n=6);

Group 6 – a group with BH 500 mg/kg·b.wt. + D-galactose 300 mg/kg·b.wt. (n=6); After one week of acclimatization, the rats were randomly divided into six groups (n = 6) and administered with various doses for 42 days. Behavior was assessed and at the end of the experimentation rat brains were removed, rinsed in with ice-cold saline then isolated and brains were fixed in 10% formalin for H & E staining. Collected brains were kept at −80 ◦C for Bio-molecular studies. [13].

6 *IN-VIVO* BEHAVIOUR PARAMETER

6.1 Actophotometer (ATPM)

ATPM test, the Locomotion Behavior of rats was observed in a brightly illuminated square-shaped arena. The animal was placed in the center and allowed to explore the open field for 5 min. [14].

6.2 Rotar-rod (RR)

In RR test animals were evaluated for grip strength and balance. Each animal was placed on the rotating rod at a speed of 25 rpm. Three separate Trials were performed for each rat at 5-minute intervals and a cutoff time of 180 seconds. The average results were recorded as a fall in time [15].

6.3 Hanging wire (HW)

The HW test was used to measure the gripping and forelimb strength of rats. The length of time the rats were able to hold the wire was recorded. The cut-off time was taken as 90 s [16].

6.4 Novel object recognition (NOR)

The NOR test was carried out for cognition deficits. The time taken by the rats to explore the two objects during the acquisition and retention phases of the test was recorded manually and separately with two stopwatches (by a trained observer). Its Discrimination Index (DI) was then calculated [17].

6.5 Forced swim (FS)

The FS test is a common test to assess depressive-like Behavior. The test consists of a training and a test session on several consecutive days. The rats were placed in the cylinder for 10 min during the training and 5 min during the test session. Movements in the cylinder were recorded. The time (s) the animals spent immobile during training and during a test were used for the analysis [18].

7 ANTIOXIDANT STUDIES

7.1 Acetylcholinesterase (AchE) content

Briefly, 0.1 ml of 0.01M DTNB was added to 2.6 ml of 0.1M phosphate buffer (pH 8.0). 0.04ml of brain tissue supernatant was added to the above mixture followed by incubation for 5min. Then 0.04ml of substrate (0.075Macetylthiocholine iodide) was added to the reaction mixture. The OD was read at continuously for 5min at 1min intervals [19].

7.2.Catalyse (CAT)

Briefly, 0.95 ml of 10 mM H2O2 60 mM phosphate buffer (pH=7.0), 50 µl of the brain tissue supernatant were added, and The OD was read at 240 nm per min [20].

$$\text{CAT (in units)} = \frac{\text{Absorbance (A-B)} \times 50}{\text{Absorbance (A)}}$$

7.3.*Glutathione (GSH)*

Briefly, 0.5 ml of the brain homogenate tissue, and 2 ml of DTNB were added and the volume was made up to 3 ml with phosphate buffer. DTNB was reduced by the sulfhydryl compounds to form a yellow-colored complex which was measured using a UV-spectrophotometer at 412 nm [21].

$$\text{GSH (in units)} = \frac{\text{Absorbance (A-B)} \times 50}{\text{Absorbance (A)}}$$

7.4.Superoxide dismutase (SOD)

Briefly,100 µl of brain tissue supernatant add 0.8 ml of carbonate buffer (pH 10.2), incubate the above solution for 15 min initiate by adding 100 µl of adrenaline solution (1 mM). Record the change in absorbance at 295 nm UV-spectrophotometer [22].

$$\text{SOD (in units)} = \frac{\text{Absorbance (A-B)} \times 50}{\text{Absorbance (A)}}$$

7.5.Advanced oxidation protein products (AOPP)

Briefly, 200ml was diluted with phosphate-buffered saline in a ratio of 1:3, and 100 ml of 1.15 M potassium iodide was added and incubated for 2 min followed by the addition of 200 ml of acetic acid. The OD was read at a 340 nm UV-spectrophotometer [23].

$$\text{AOPP (in units)} = \frac{\text{Absorbance (A-B)} \times 50}{\text{Absorbance (A)}}$$

7.6.Malondialdehyde (MDA)

Briefly, 1ml of the above brain tissue supernatant with 2ml of TCATBA-HCL was mixed thoroughly and heated for about 30min in a boiling water bath then cooled in an ice bath for 10min followed by centrifugation at 6000rpm for 10min. The OD was read at a 532nm UV-spectrophotometer [24].

$$\text{MDA (in units)} = \frac{\text{Absorbance at 532 nm} \times 105}{1.56}$$

7.7.Glutathione S-Transferase (GST)

Briefly, the brain tissue supernatant was precipitated with 4% sulfosalicylic acid in a ratio of 1:1. The samples were kept at 4^0C for 1 h and then subjected to centrifugation at 5000 rpm for 10 min at 4 C. The assay mixture consisted of 550 ml of 0.1 M phosphate buffer, 100 ml of supernatant and 100 ml of DTNB. The OD was read at 412 nm [24]

$$\text{GST (in units)} = \frac{\text{Absorbance (A-B)} \times 50}{\text{Absorbance (A)}}$$

7.8.Estimation of Total thiol (TT)

Briefly, 0.2 ml of the tissue sample, 1.5 ml of sodium phosphate buffer (0.08 M; pH 8), 0.5 mg/ml of EDTA, and 2% sodium dodecyl sulfate were added. After vertexing, 0.1 ml of 0.01% DTNB in sodium phosphate buffer (0.1 M; pH 8) was added and mixed. This was incubated at room temperature for 15 min and The OD was read at 680nm on a UV-spectrophotometer [24].

$$\text{TT (in units)} = \frac{\text{Absorbance (A-B)} \times 50}{\text{Absorbance (A)}}$$

7.9.Estimation of Nitric oxide (NO)

Briefly, 0.5 ml of each brain tissue supernatant was taken for the test.0.5 ml of the Sulphanilamide Solution was dispensed into the respective test tubes containing all the brain tissue supernatant and varied concentrations of Nitrite Standard solutions (100, 50, 25, 12.5, 6.25, 3.13, and 1.56 µm). Incubated for 5-10 min at room temperature and protected from light. Using a pipette, 0.5 ml of the NED Solution was dispensed to all test tubes. A purple/magenta color will begin to form immediately. The OD was read at 535 nm using UV-spectroscopy. [24].

$$\text{NO (in units)} = \frac{\text{Absorbance (A-B)} \times 50}{\text{Absorbance (A)}}$$

7.10.Estimation of Glutathione Peroxidases (GPx)

Glutathione Peroxidases were based on the measures of the enzymatic reduction of H_2O_2 by GPx through consumption of reduced glutathione (GSH) that is restored from oxidized glutathione GSSG in a coupled enzymatic reaction by GR. GR reduces GSSG to GSH using NADPH as a reducing agent. The decrease in OD was read at was measured in a UV-spectrophotometer [24].

$$GPx\ (\text{in units}) = \frac{\text{Absorbance (A-B)} \times 50}{\text{Absorbance (A)}}$$

7.11.Hippocampus preparations

The hippocampus of each rat was dissolved in ice and immersed in ice-cold physiological saline. The brain tissue supernatant was collected after centrifugation at 3500 rpm for 10 min at 4^0C for various biochemical assays [25].

7.12.Western-blotting

The 100-200 mg tissue harvested for the assay, was washed tissue thoroughly in cold PBS and resuspended tissue in 300 µl of ice-cold RIPA buffer. Homogenized tissue with a homogenizer until a sample is fully homogenized and is completely smooth. The samples were incubated for 30 mins by gentle mixing every 5 mins at 4°C. Centrifuged the sample at 10,000 x g for 15 minutes at 4°C in a cold centrifuge and supernatant was collected and transferred to a clean tube on ice. Determined the sample protein concentration by extracting a portion of the sample.

7.13..SDS-PAGE

The 50-µg protein sample from each cell lysate was mixed with 5X loading dye and heated for 2 mins at 95°C. Protein samples were loaded and separated on 12 % and 15% SDS-PAGE gel using Mini protean Tetra cell (Bio-Rad). Nitrocellulose membrane (0.2 µM) was equilibrated in transfer buffer for 10 mins at RT. Protein transfer was done for 15 mins in Turbo Transblot (Bio-Rad) apparatus at 2.5 A and 25 V. Blot was blocked in 3% BSA in TBST for 1hr at RT followed by incubation with respective 1° Ab at appropriate dilutions overnight at 4°C. The blot was washed thrice with TBST for 5 mins at RT. The blot was incubated with 2° Ab (anti-Rabbit or anti-Mouse IgG- HRP) at dilution 1:10000 for 1 hr. at RT. Washed 3 times with TBST for 5 mins at RT. The blot was rinsed with ECL reagent (two-component system) for 1 min in the dark and the image was captured between 0.5 sec to 15 secs exposure in Chemidoc XRS+ imaging system (Bio-Rad).

8. Results and Discussion

8.1. *In-vivo* studies

8.2.Actophotometer

In D-gal alone treated rats showed a significant ($^{###}p<0.001$) decrease in locomotor activity when compared to the normal control group. when treated with BH treatments show ($^{**}p<0.01$, $^{***}p<0.001$) when compared with the normal group, and Donepezil significantly shows ($^{***}p<0.001$) preserved locomotor activity score. Results are summarized in Table 1.

Groups	1st consecutive week	3rd consecutive week	5th consecutive week
Control	138.63 ± 0.77	135.50±0.61	141.33±0.92
Donepezil 2mg/kg (Standard)	192.93 ±3.35***	184.57±2.69***	185.90±1.71***
D-gal 300mg/kg (Inducer)	89.97±1.21###	76.30±0.72###	67.13±0.50###
BH 150mg/kg	178.53±0.56**	188.83±0.50***	136.50±3.85**
BH 250mg/kg	181.20±1.20***	192.30±0.74**	197.00± 0.81***
BH 500mg/kg	187.53±0.58***	195.50±0.76**	192.77±0.56**

Table 1. Statistical analysis data are expressed by one-way ANOVA followed by Tukey's test. $^{#}p<0.05$, $^{##}p<0.01$, $^{###}p<0.001$ compared to the normal control group and $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to the D-gal model group.

8.3.Rotar Rod

]In D-gal alone treated rats showed a significant ($^{###}p<0.001$) drop in motor coordination when compared to the normal control group. when treated with BH treatments shows restored motor coordination ($^{**}p<0.01$, $^{***}p<0.001$) when compared with the normal group and Donepezil significantly shows ($^{***}p<0.001$) conserved motor coordination. Results are summarized in Table 2.

Groups	1st consecutive week	3rd consecutive week	5th consecutive week
Control	91.6±0.70	94.93±0.42	94.90±0.38
Donepezil 2mg/kg (Standard)	90.1±0.67***	94.93±0.59***	96.63±0.37***
D-gal 300mg/kg (Inducer)	86.1±0.96###	72.80±0.4###	64.20± 0.54###
BH 150mg/kg	82.9±1.11**	87.57±0.57**	91.73±0.43***
BH 250mg/kg	81.5±0.43***	84.60±0.38***	85.47±0.25**
BH 500mg/kg	84.0±0.75***	85.83±0.42**	87.50±0.41**

Table 2. Statistical analysis data are expressed by one-way ANOVA followed by Tukey's test. #$p<0.05$, ##$p<0.01$, ###$p<0.001$ compared to the normal control group and *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to the D-gal model group.

8.4.Grip Strength

In D-gal alone treated rats showed a significant (###$p<0.001$) decrease in muscle strength and motor coordination when compared to the normal control group. when treated with BH treatments shows brought back muscle strength and motor coordination (**$p<0.01$, ***$p<0.001$) when compared with the normal group, and Donepezil significantly shows (***$p<0.001$) well-maintained muscle strength and motor coordination. Results are summarized in Table 3

Groups	1st consecutive week	3rd consecutive week	5th consecutive week
Control	140.83±0.57	132.17±0.52	134.03±0.39
Donepezil 2mg/kg (Standard)	128.50±0.53***	130.60±0.41***	131.90±0.34***
D-gal 300mg/kg (Inducer)	121.47±0.39###	90.93±0.59###	73.80±0.41###
BH 150mg/kg	115.37±0.70***	119.57±0.35***	121.53±0.37***
BH 250mg/kg	114.77±0.40**	117.80±0.63**	120.93±0.57**
BH 500mg/kg	116.13±0.29**	118.47±0.45**	120.47±0.39**

Table 3. Statistical analysis data are expressed by one-way ANOVA followed by Tukey's test. #$p<0.05$, ##$p<0.01$, ###$p<0.001$ compared to the normal control group and *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to the D-gal model group.

8.5.Time Spent Near Novel Object

The D-gal group showed a significant (###$p<0.001$) deficit in object recognition learning compared with the control group. In contrast, the rats with BH extract showed a significant (*$p<0.05$, **$p<0.01$, ***$p<0.001$) improvement in learning and memory in the object-placed recognition task. these results suggest that BH administration can significantly ameliorate the memory ability of D-gal induced aged rats for time spent near novel objects and discrimination index. Results are summarized in Tables 4 & 5.

Groups	1st consecutive week	2nd consecutive week	3rd consecutive week	4th consecutive week	5th consecutive week
Control	195.07±0.98	184.60±0.86	181.30±1.27	185.53±0.92	193.13±1.20
Donepezil 2mg/kg (Standard)	177.83±0.93***	168.23±0.83***	157.00±0.56***	163.83±0.67***	188.90±0.76***
D-gal 300mg/kg	180.20±0.8###	164.20±0.67###	142.43±0.66###	135.27±0.78###	126.07±1.15###

(Inducer)					
BH 150mg/kg	178.97±2.23***	165.43±1.25***	171.23±0.50**	165.33±0.99**	170.43±1.34*
BH 250mg/kg	170.07±1.44**	174.20±1.12**	178.10±0.83**	184.57±0.82**	188.47±1.13**
BH 500mg/kg	185.37±1.06**	180.50±1.38**	189.70±0.4**	191.63±0.60**	195.70±0.92***

Table 4. Statistical analysis data are expressed by one-way ANOVA followed by Tukey's test. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to the normal control group and $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to the D-gal model group.

8.6.Discrimination index

Groups	1st consecutive week	2nd consecutive week	3rd consecutive week	4thconsecutive week	5th consecutive week
Control	0.69±0.13	0.68±0.09	0.69±0.10	0.63±0.12	0.59±0.09
Donepezil 2mg/kg (Standard)	0.58±0.12***	0.67±0.04***	0.55±0.08***	0.60±0.09***	0.61±0.07***
D-gal 300mg/kg (Inducer)	0.55±0.05###	0.43±0.06###	0.50±0.13###	0.37±0.05###	0.26±0.05###
BH 150mg/kg	0.47±0.05***	0.53±0.05***	0.61±0.13***	0.54±0.07**	0.45±0.06*
BH 250mg/kg	0.63±0.06***	0.61±0.08**	0.63±0.12***	0.63±0.06**	0.64±0.09**
BH 500mg/kg	0.72±0.0***	0.72±0.04**	0.74±0.0**	0.69±0.06**	0.75±0.06***

Table 5. Statistical analysis data are expressed by one-way ANOVA followed by Tukey's test. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to the normal control group and $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to the D-gal model group.

9.1.Force swim

9.1.1.Escape Time Latency (ETL)

The D-gal treated rats had impaired cognition and memory as indicated by the ETL across the successive days ($^{\#\#\#}p<0.001$) compared with a control group and spent a significantly decreased time in the goal area (**p<0.05) compared with those of the control group during the test hidden sessions. The D-gal treatment plus BH administration group displayed remarkable increases in the time spent in the goal area ($^{***}p<0.001$), and reduced escape latencies in the probe test (**p< 0.05) compared to those of the model group. These results indicate that the D-gal treated rats had impaired spatial learning and memory, while the administrated extracts could recover the aged-related cognitive impairment caused by D-gal. Results are summarized in Tables 6 & 7.

Groups	1st consecutive week	2nd consecutive week	3rd consecutive week	4th consecutive week	5th consecutive week
Control	88.17±0.50	77.80±0.38	68.47±1.24	62.17±1.42	62.17±1.42
Donepezil 2mg/kg (Standard)	70.74±0.57***	67.40±0.72***	60.80±0.38***	52.93±0.60***	44.13±0.58***
D-gal 300mg/kg (Inducer)	86.33±0.45###	64.47±0.64###	51.80±0.63###	42.53±0.44###	64.47±0.59###

BH 150mg/kg	78.30±0.23***	75.50±1.25**	46.93±0.42**	30.10±0.74**	26.63±0.83**
BH 250mg/kg	71.23±0.55***	67.87±0.81***	37.43±1.32*	42.20±0.56*	28.50±0.91**
BH 500mg/kg	60.83±0.57***	53.00±0.51**	27.53±0.63**	22.53±0.70*	19.87±0.47*

Table 6. Statistical analysis data are expressed by one-way ANOVA followed by Tukey's test. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to the normal control group and $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to the D-gal model group.

9.1.2.Time Spent in Target Quadrant

Groups	1st consecutive week	2nd consecutive week	3rd consecutive week	4th consecutive week	5th consecutive week
Control	79.10±0.52	74.43±1.26	76.77±1.04	80.80±1.26	81.77±1.22
Donepezil 2mg/kg (Standard)	69.67±0.80***	60.17±0.72***	72.20±0.59***	85.83±0.94***	92.17±0.74***
D-gal 300mg/kg (Inducer)	75.53±1.02###	64.20±0.85###	58.00±1.50###	46.97±1.11###	20.60±0.68###
BH 150mg/kg	86.30±0.48***	70.80±0.55**	75.63±0.70***	49.37±0.84**	56.90±0.78**
BH 250mg/kg	76.63±0.82***	59.77±1.02**	63.83±0.95**	32.93±1.17**	46.97±1.11*
BH 500mg/kg	75.17±0.98***	54.53±1.23**	41.53±0.71**	29.73±0.94**	27.03±0.68**

Table 7. Statistical analysis data are expressed by one-way ANOVA followed by Tukey's test. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to the normal control group and $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to the D-gal model group.

9.2.*In-vitro* AchE content

The D-gal group showed a significant ($^{\#\#\#}p<0.001$) increase in AchE level in hippocampus tissues compared with the control group. BH extract administration showed significantly reversed abnormalities in AchE activities compared with those of the control group (Fig. 2). This result indicated that BH extract might ameliorate dysfunction of the cholinergic system.

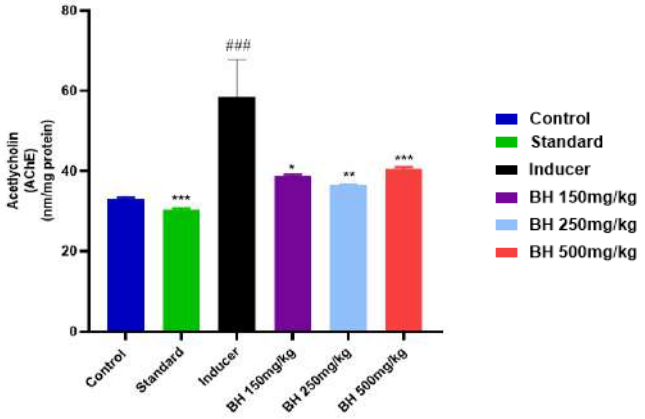

Table 2. Statistical analysis data are expressed by one-way ANOVA followed by Tukey's test. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to the normal control group and $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to the D-gal model group. Note: C) Control group; STD) donepezil 2mg/kg; I) D-gal 300mg/kg; BH) Low-dose of BH 150mg/kg; BH) Middle-dose of BH 250mg/kg; BH) High-dose of BH 500mg/kg extract group.

9.2.1.Antioxidant studies

D-gal treatment caused notable oxidative damage, including a decrease in CAT, GSH-Px, SOD, Total thiol, GPx, and AOPP, as well as an increase in AchE, MDA, GST, and NO levels in the hippocampus of rats, which was similar to the previous report [33]. BH treated with extracts significantly reserved the changes in CAT, GSH-Px, SOD, Total thiol, GPx, AOPP, and MDA levels, suggesting that the cognitive effect of BH scavenged ROS mainly by enhancing antioxidant activity and consequently decreasing lipid peroxidative damage. Moreover, we observed that accompanied by the increase of CAT, GSH-Px, SOD, Total thiol, GPx, and AOPP levels in the rat's hippocampal region. NO was significantly decreased by BH treatment. This finding indicated that BH mobilized its protective mechanism to inhibit the overproduction of NO in the rat's hippocampus region. graphical representation of results is represented in the figure. 3.

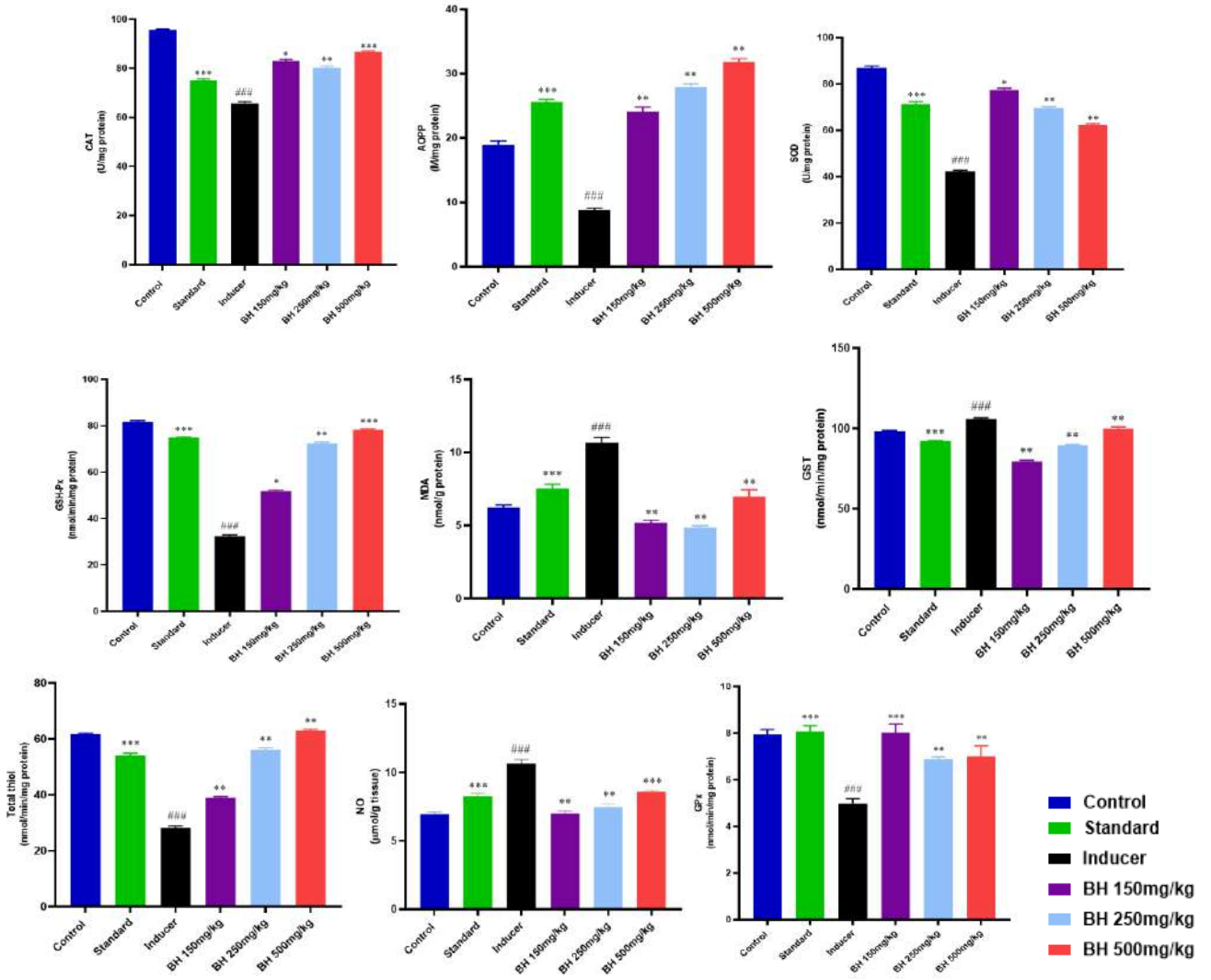

Fig 3. Statistical analysis data are expressed by one-way ANOVA followed by Tukey's test. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to the normal control group and $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to the D-gal model group. Note: C) Control group; STD) donepezil 2mg/kg; I) D-gal 300mg/kg; BH) Low-dose of BH 150mg/kg; BH) Middle-dose of BH 250mg/kg; BH) High-dose of BH 500mg/kg extract group.

9.2.2.H & E staining

In Fig. 4. Pyramidal neuron shrinkage and chromatin condensation of nuclei were also observed in D-gal group. Treatment with BH results in an improvement in the structure of these neurons. Rats in the control group had full hippocampal neurons, which were arranged tightly and were morphologically intact. The pyramidal cells neurons presented round and large nuclei and clean nucleoli (Fig. 4.C & 4.STD). Widespread damage was visible in the hippocampi of the D-gal treated group (Fig. 4I). The intercellular space increased in size, and the cells were loosely arranged. The pyramidal neurons either presented a densely stained shrunken appearance with minimal cytoplasm or disappeared. However, the Administration of BH extract was after the D-gal was injected, the neuronal cells in the hippocampus were rescued (Fig. 4Lb-hb) compared with those of the D-gal. the neurons in the hippocampi of the rats treated with BH at the dose of 500mg/kg of appeared almost normal (Fig. hb).

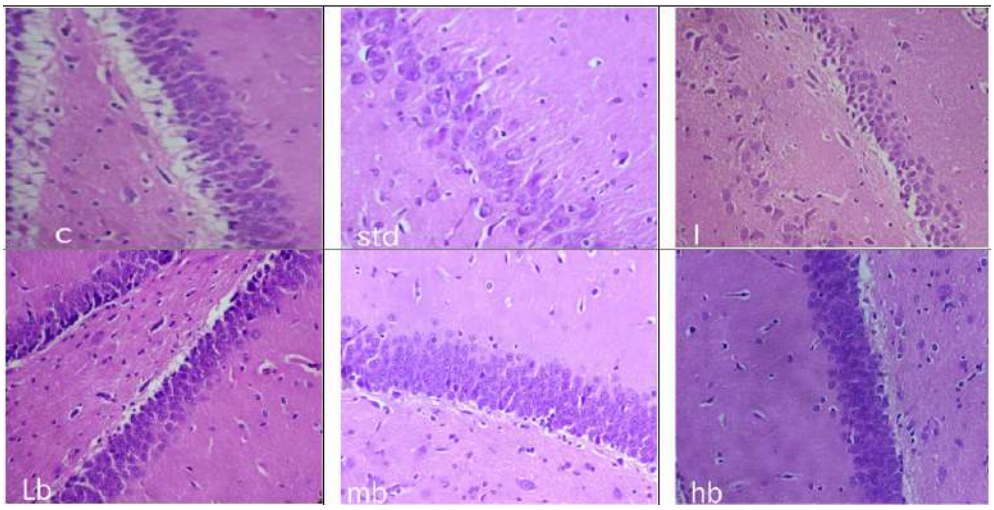

Fig 4. The protective effect of BH on the cell loss in the hippocampi of D-gal treated rats was revealed by H&E staining (×200). Note: C) Control group; STD) donepezil 2mg/kg; I) D-gal 300mg/kg; Lb) Low-dose of BH 150mg/kg; mb) Middle-dose of BH 250mg/kg; hb) High-dose of BH 500mg/kg extract group.

9.2.3.H & E staining of Organ

The tissues of the kidney, liver, spleen, and heart were observed in Fig. 5. These tissues in the control group showed cardiac muscle fiber cell bodies extending to a shuttle shape, a trend of parallel assembling into a beam, structure integrated, intercellular boundaries clear, packing closely, graduation distinct, clearly visible band, and intercalated disc. However, these tissues in the aging model group showed cardiac muscle fiber plumping, structure fuzzy and twisted shortening, significantly widened interval, and obvious capillary vessels of myocardial interstitial congestion. After BH treatment, the microstructures of the rat's viscera were obviously improved, which was similar to those of the Vc group. These results indicated that D-gal induced changes in the morphology, number of hepatocytes, and neurons. Meanwhile, the BH extracts have protective effects on the microstructure of rat viscera.

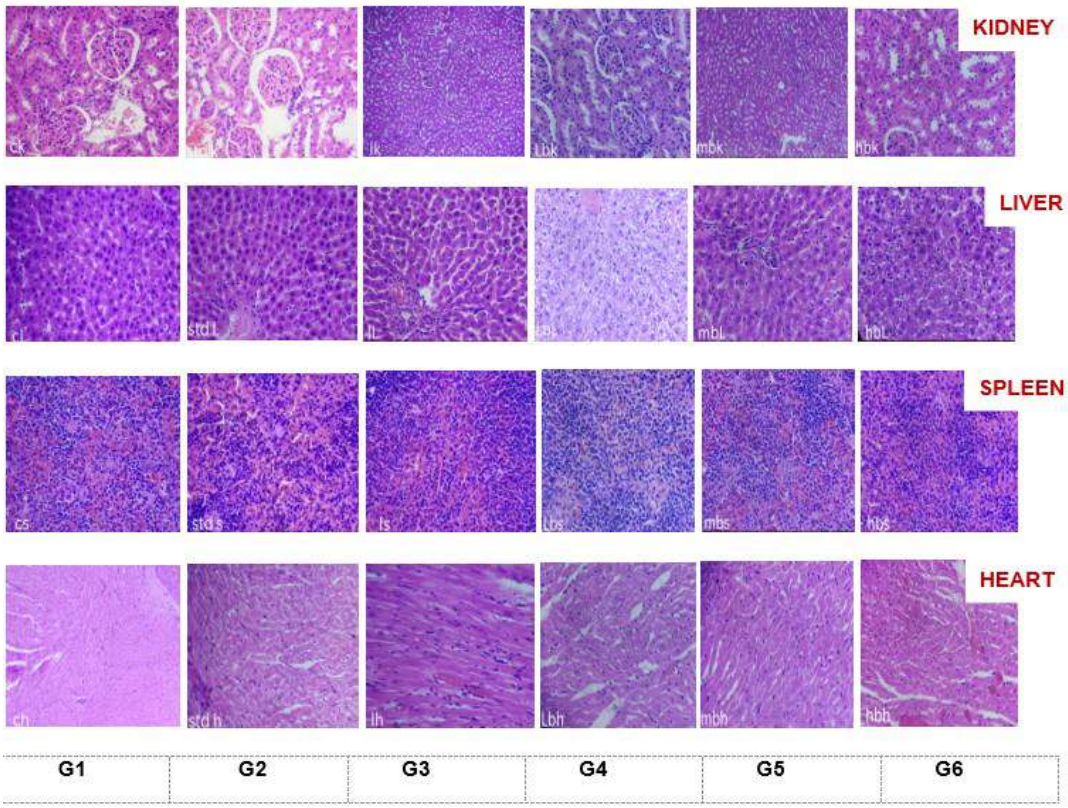

Fig 5. The Organs tissues stained with H & E obtained from D-galactose-induced in rats: G1- Control group, G2- Donepezil 2mg/kg; G3 $_{-D}$-gal 300mg/kg; G4- Low-dose of BH 150mg/kg; G5- Middle-dose of BH 250mg/kg; G6- BH 500mg/kg extract group.

9.2.4.Crystel Violet

As shown in Fig.6, the observation of the control group showed cells with well-defined nuclear membranes, clearly visible nucleolus, and fewer abnormalities. Noticeable changes were observed in the $_{D}$-gal, which included cells with indistinct nuclear membrane as well as no prominent nucleolus, besides being darkly stained. Further, the number of normal cells was also reduced in the hippocampus region in the $_{D}$-gal group. Interestingly, these pathological changes observed in the hippocampus of a $_{D}$-gal group rats were altered in groups where Donepezil 2mg/kg·b.wt. or BH at doses of 150, 250, and 500 mg/kg·b.wt. the results indicated that BH showed a protective effect.

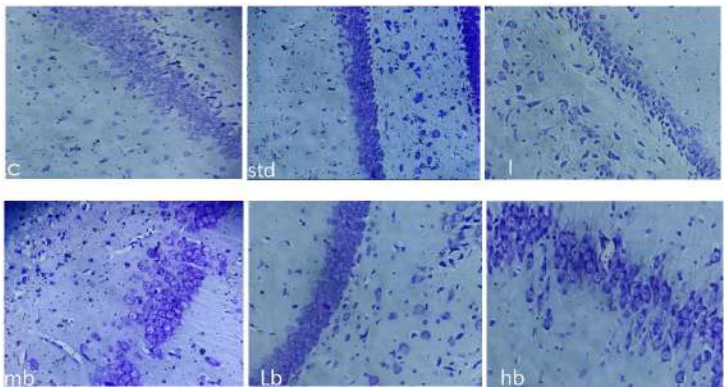

Fig 6. The protective effect of BH on cell loss in the hippocampi of $_{D}$-gal treated rats was revealed by Crystel violet staining (×200). Note: C) Control group; STD) donepezil 2mg/kg; I) $_{D}$-gal 300mg/kg; Lb)

Low-dose of BH 150mg/kg; mb) Middle-dose of BH 250mg/kg; hb) High-dose of BH 500mg/kg extract group.

9.2.5.Western blotting

Bcl-2 is an anti-apoptotic protein, and Bax pro-apoptotic proteins and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is one of the most commonly used housekeeping genes used in comparisons of gene expression data [26-27]. To further explore the cognitive mechanism of BH in D-gal treated rats, a western blot was used to detect the protein levels of Bax, Bcl-2 in the hippocampus. The western blot analysis revealed that D-gal led to a significant increase in the Bax/Bcl-2ratio (Fig. 7A and B), levels in the model group compared with those of the control group. BH extract administration remarkably reduced the Bax/Bcl-2 ratio, and the levels compared with those of the D-gal treated rats. These results indicate that BH extract has the effects of anti-apoptosis and neuroprotection through regulating the expression of apoptosis-related proteins. The western blot analysis also showed that BH extracts have a neuroprotective effect on the rat aging model induced by D-gal.

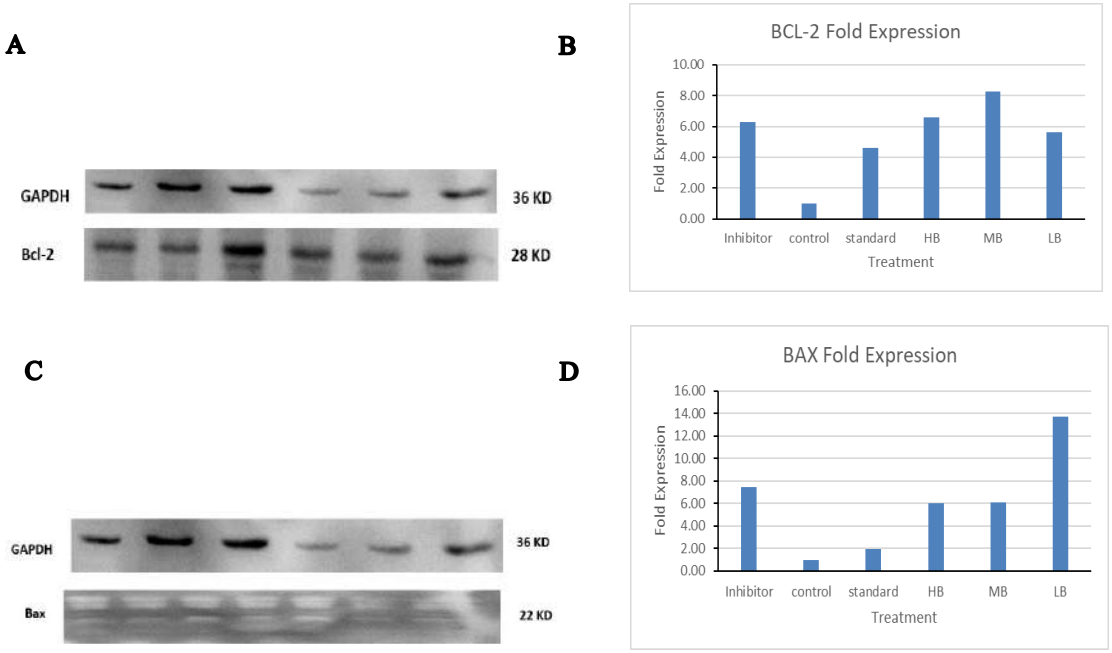

Fig 7.A: The band are from a representative blot. Loading details: Lane 1: Inhibitor (D-gal 300mg/kg); Lane 2: Control group; Lane 3: Standard (Donepezil 2mg/kg); Lane 4: HB 500mg/kg; Lane 5: MB (Middle-dose of BH 250mg/kg); Lane 6: LB (Low-dose of BH 150mg/kg).

CONCLUSION

The findings of this study suggest that BH seed extract possesses potential aging properties against cognitive impairment in aged rats. This effect may be attributed to its ability to alleviate oxidative stress and neurodegeneration. Further research addressing the underlying mechanisms and clinical trials are warranted to validate the efficacy of BH as a therapeutic option for age-related cognitive impairment.

Ethics Statement

The study was carried out in accordance with CPCSEA guidelines. The protocol was approved by the Institutional Animal Ethics Committee (approval no. SACCP-IAEC/2022-02/69).

Acknowledgment

I would like to show gratitude to my research guide Dr. Bharathi, HOD & Professor Sri Adichunchanagiri College of Pharmacy, B.G. Nagara-571448.

Conflict of interest: the authors declare that they have no conflicts of interest.

Financial support: the authors declare that they have no financial support.

REFERENCES

1. Li XL, Tao X, et al.Cognitive-exercise dual-task intervention ameliorates cognitive decline in natural aging rats through reducing oxidative stress and enhancing synaptic plasticity. Exp Gerontol. 2022 Nov; 169:111981. doi: 10.1016/j.exger.2022.111981. Epub 2022 Oct 18. PMID: 36270545.
2. Mani V, Rashed Almutairi S. Impact of levetiracetam on cognitive impairment, neuroinflammation, oxidative stress, and neuronal apoptosis caused by lipopolysaccharides in rats. Saudi Pharm J. 2023 Sep;31(9):101728. doi: 10.1016/j.jsps.2023.101728. Epub 2023 Aug 3. PMID: 37583755; PMCID: PMC10424214.
3. Malhotra RK, Manoharan N, Deo SS. The Trend and Prediction of Cervical Cancer Incidence in Delhi, India: An Age-Period-Cohort Analysis. Asian Pac J Cancer Prev. 2022 Aug 1;23(8):2787-2795. doi: 10.31557/APJCP.2022.23.8.2787. PMID: 36037135; PMCID: PMC9741874.
4. Umbayev B, Askarova S, Almabayeva A, Saliev T, Masoud AR, Bulanin D. Galactose-Induced Skin Aging: The Role of Oxidative Stress. Oxid Med Cell Longev. 2020 Jun 17;2020:7145656. doi: 10.1155/2020/7145656. PMID: 32655772; PMCID: PMC7317321.
5. He X, Yan C, et al.vThe preventive effects of probiotic Akkermansia muciniphila on D-galactose/AlCl3 mediated Alzheimer's disease-like rats. Exp Gerontol. 2022 Dec;170:111959. doi: 10.1016/j.exger.2022.111959. Epub 2022 Sep 21. PMID: 36152776.
6. Gill V, Kumar V, Singh K, Kumar A, Kim JJ. Advanced Glycation End Products (AGEs) May Be a Striking Link Between Modern Diet and Health. Biomolecules. 2019 Dec 17;9(12):888. doi: 10.3390/biom9120888. PMID: 31861217; PMCID: PMC6995512.
7. Zhang SJ, Su SH, Gao JF. Electroacupuncture improves learning-memory ability possibly by suppressing apoptosis and down-regulating expression of apoptosis-related proteins in hippocampus and cerebral cortex in immature mice with Alzheimer's disease. Zhen Ci Yan Jiu. 2020 Aug 25;45(8):611-6. Chinese. doi: 10.13702/j.1000-0607.200080. PMID: 32869569.
8. Zhang N, Gordon ML. Clinical efficacy and safety of donepezil in the treatment of Alzheimer's disease in Chinese patients. Clin Interv Aging. 2018 Oct 11;13:1963-1970. doi: 10.2147/CIA.S159920. PMID: 30349215; PMCID: PMC6187917.
9. Islam MT, Quispe C, et al. A Literature-Based Update on Benincasa hispida (Thunb.) Cogn.: Traditional Uses, Nutraceutical, and Phytopharmacological Profiles. Oxid Med Cell Longev. 2021 Dec 10;2021:6349041. doi: 10.1155/2021/6349041. PMID: 34925698; PMCID: PMC8683187.
10. Yusoff IM, Chua LS, Taher ZM. Valorization of fruit waste from Cucurbitaceae family: Profiling of phytoconstituent of Benincasa hispida and Citrullus lanatus rinds using ultrasound-assisted extraction. Food Bioscience. 2023 Feb 1;51:102190.
11. Tozlu ÖÖ, Türkez H, et al. Assessment of the neuroprotective potential of d-cycloserine and l-serine in aluminum chloride-induced experimental models of Alzheimer's disease: In vivo and in vitro studies. Frontiers in Nutrition. 2022 Sep 8;9:981889.
12. Shakya A, Chaudhary SK, et al. Acute and sub-chronic toxicity studies of Benincasa

hispida (Thunb.) cogniaux fruit extract in rodents. Regul Toxicol Pharmacol. 2020 Dec;118:104785. doi: 10.1016/j.yrtph.2020.104785. Epub 2020 Sep 22. PMID: 32976857.

13. Feng W, Liu J, et al. Alginate oligosaccharide alleviates D-galactose-induced cardiac ageing via regulating myocardial mitochondria function and integrity in mice. J Cell Mol Med. 2021 Aug;25(15):7157-7168. doi: 10.1111/jcmm.16746. Epub 2021 Jul 6. PMID: 34227740; PMCID: PMC8335675.
14. Tiwari A, Khera R, et al.Neuroprotective Effect of α-Mangostin in the Ameliorating Propionic Acid-Induced Experimental Model of Autism in Wistar Rats. Brain Sci. 2021 Feb 25;11(3):288. doi: 10.3390/brainsci11030288. PMID: 33669120; PMCID: PMC7996534.
15. Rakhunde PB, Saher S, Ali SA. Neuroprotective effect of Feronia limonia on ischemia reperfusion induced brain injury in rats. Indian J Pharmacol. 2014 Nov-Dec;46(6):617-21. doi: 10.4103/0253-7613.144920. PMID: 25538333; PMCID: PMC4264077.
16. Adebiyi OE, Omobowale TO, Abatan MO. Neurocognitive domains and neuropathological changes in experimental infection with Trypanosoma brucei in Wistar rats. Heliyon. 2021 Oct 26;7(11):e08260. doi: 10.1016/j.heliyon.2021.e08260. PMID: 34765779; PMCID: PMC8571699.
17. Wang Q, Dong L, et al. Dammarane Sapogenins Improving Simulated Weightlessness-Induced Depressive-Like Behaviors and Cognitive Dysfunction in Rats. Front Psychiatry. 2021 Mar 26;12:638328. doi: 10.3389/fpsyt.2021.638328. PMID: 33841208; PMCID: PMC8032884.
18. Kelberman MA, Anderson CR, et al. Consequences of Hyperphosphorylated Tau in the Locus Coeruleus on Behavior and Cognition in a Rat Model of Alzheimer's Disease. J Alzheimers Dis. 2022;86(3):1037-1059. doi: 10.3233/JAD-215546. PMID: 35147547; PMCID: PMC9007891.
19. Arya A, Chahal R, et al.Acetylcholinesterase Inhibitory Potential of Various Sesquiterpene Analogues for Alzheimer's Disease Therapy. Biomolecules. 2021 Feb 25;11(3):350. doi: 10.3390/biom11030350. PMID: 33669097; PMCID: PMC7996600.
20. Jiang W, YanYu, et al. Single Point Mutation from E22-to-K in Aβ Initiates Early-Onset Alzheimer's Disease by Binding with Catalase. Oxid Med Cell Longev. 2020 Dec 24;2020:4981204. doi: 10.1155/2020/4981204. PMID: 33425208; PMCID: PMC7775154.
21. Chaâbane M, Koubaa M, et al. N. Nitraria retusa fruit prevents fluconazole-induced kidney injury in adult rats through modulation of oxidative stress and histopathological changes. Pharm Biol. 2017 Dec;55(1):1061-1073. doi: 10.1080/13880209.2016.1278455. PMID: 28198206; PMCID: PMC6130529.
22. Zhang C, Rodriguez C, et al. Age-dependent and tissue-related glutathione redox status in a mouse model of Alzheimer's disease. J Alzheimers Dis. 2012;28(3):655-66. doi: 10.3233/JAD-2011-111244. PMID: 22045490; PMCID: PMC4221633.
23. Pang K, Jiang R, et al.An App knock-in rat model for Alzheimer's disease exhibiting Aβ and tau pathologies, neuronal death and cognitive impairments. Cell Res. 2022 Feb;32(2):157-175. doi: 10.1038/s41422-021-00582-x. Epub 2021 Nov 17. PMID: 34789895; PMCID: PMC8807612.
24. Kaur A, Jaiswal G, Brar J, Kumar P. Neuroprotective effect of nerolidol in traumatic brain injury associated behavioural comorbidities in rats. Toxicol Res (Camb). 2021 Jan 5;10(1):40-50. doi:

10.1093/toxres/tfaa100. PMID: 33613971; PMCID: PMC7885190.

25. Abhishek M, Rubal S, Rohit K, Rupa J, Phulen S, Gurjeet K, Raj SA, Manisha P, Alka B, Ramprasad P, Bikash M. Neuroprotective effect of the standardised extract of Bacopa monnieri (BacoMind) in valproic acid model of autism spectrum disorder in rats. J Ethnopharmacol. 2022 Jul 15;293:115199. doi: 10.1016/j.jep.2022.115199. Epub 2022 Mar 26. PMID: 35346813.
26. Aslan A, Beyaz S, Gok O, Parlak G, Can MI, Agca CA, Ozercan IH, Parlak AE. Royal jelly protects brain tissue against fluoride-induced damage by activating Bcl-2/NF-κB/caspase-3/caspase-6/Bax and Erk signaling pathways in rats. Environ Sci Pollut Res Int. 2023 Apr;30(17):49014-49025. doi: 10.1007/s11356-023-25636-y. Epub 2023 Feb 10. PMID: 36759409.
27. Cornett K, Puderbaugh A, Back O, Craven R. GAPDH in neuroblastoma: Functions in metabolism and survival. Front Oncol. 2022 Oct 4;12:979683. doi: 10.3389/fonc.2022.979683. PMID: 36267982; PMCID: PMC9577191.

HOW TO CITE: Pooja R. C., Dr. Bharathi D. R, Benincasa Hispida Reversed D-galactose-induced oxidative Stress and neurodegeneration-mediated Cognitive Impairment in Aged Rats, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 10, 150-164. https://doi.org/10.5281/zenodo.10014344