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Beta vulgaris L. var. cicla improves memory deficits in intracerebroventricular streptozotocin injected rats: Role on neuroinflammation

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ABSTRACT: Alzheimer's disease is a challenging disease for patients due to progressive loss of cognition and behavioral disorders. Disruption of cholinergic transmission and neuroinflammation are the most important mechanisms underlying cognitive damage. *Beta vulgaris* L. var. cicla (BV) has been reported to have various pharmacological effects associated with its rich antioxidant content. In addition, anticholinesterase and anti-inflammatory activities of BV have been demonstrated in vitro. The aim of this study is to elucidate the therapeutic effect of BV against cognitive impairment, reduction in cholinergic transmission and neuroinflammation caused by intracerebroventricular (ICV) administration of streptozotocin (STZ). STZ was administered bilaterally at a dose of 3 mg/kg via ICV to rats, and BV treatment at a dose of 2 g/kg for 21 days was administered orally to STZ-induced animals. After behavioral tests, AChE activity, TNF-α and IL-1β levels were measured in hippocampus and cortex tissues excised from decapitated animals. Novel object recognition and passive avoidance test showed that the treatment of BV reverted the ICV-STZ induced memory dysfunctions in rats. Furthermore, increased AChE levels in the hippocampal and cortical tissues of STZ-induced rats were significantly reduced with 21 days of BV treatment. In conclusion, these results confirm that STZ administration caused cholinergic hypofunction, neuronal inflammation and cognitive dysfunction in rats, and BV therapy significantly inhibited these changes with its potential neuroprotective activity.

KEYWORDS: Alzheimer's disease; *Beta vulgaris* L. var. cicla; cholinergic dysfunction; neuroinflammation; cognitive function.

1. INTRODUCTION

Alzheimer's disease (AD), one of the most common forms of dementia worldwide, has been defined as progressive multifarious cognitive and behavioral deterioration [1]. Increasing evidence indicates that accumulation of extracellular amyloid plaques and formation of intracellular neurofibrillary tangles which constitute the main pathologies of the AD, stimulate destruction of cholinergic neurons by inducing neuroinflammation [2]. Since cognitive functions are provided by impulse transmission of acetylcholine in neurons [3], acetylcholinesterase (AChE) enzyme, which metabolizes acetylcholine, has a fundamental role in the regulation of cholinergic transmission in the hippocampus and cerebral cortex [4-6]. A growing body of evidence suggests that the AChE enzyme both triggers deposition of amyloid and formation of plaque and enhancing in neurofibrillary tangles [7]. Thus, it causes neuronal dysfunction in AD pathology not only by inhibiting cholinergic transmission but also by causing amyloid aggregation [8]. Many studies have shown that neuroinflammation occurring in the early stages of AD has been one of the main factors in the course of the disease due to its effects on amyloid deposition, tangle formation, tau phosphorylation and neuronal loss [9]. Under the influence of inflammation, the activation of microglia and the increase of proinflammatory

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cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) cause amyloid plaque accumulation and loss of cholinergic neurotransmission, impairing cognitive functions in AD [10]. Based on this knowledge, several therapeutic strategies are under investigation to inhibit neuroinflammation and increase cholinergic transmission for amelioration of memory and cognitive disturbance.

According to the literature, intracerebroventricular (ICV) injection of a sub-diabetogenic dose of streptozotocin (STZ) induces behavioral and neuropathological changes like sporadic AD in experimental animals [11]. STZ causes progressive memory loss by disrupting glucose metabolism, causing inflammation, and leading to neuronal death. Decreased brain insulin sensitivity forms the background of the pathogenesis of familial and sporadic of AD [12]. Therefore, it is widely preferred as an applicable and demonstrative method as an experimental AD model suitable for rodents in pharmacology studies [13]. The partial therapeutic effects and side effects of NMDA antagonist (memantine) and cholinesterase inhibitors (eg galantamine, rivastigmine), which are among the current palliative treatment options, necessitate the search for safe and natural new therapeutic agents [14].

Beta vulgaris L. var. cicla (BV), commonly known as Swiss chard, sea beet, foliage beet or leaf beet grows wild in Egypt and some inland sites in the Mediterranean region [15]. Numerous studies in recent years have shown that this plant extract has beneficial properties for health with its antioxidant, antibacterial, hepatoprotective, hypocholesteremic, anticoagulant activity [16, 17]. Bolkent et al. [16] reported the hypoglycemic effect of BV treatment in the model of type-2 diabetes in rats. In addition, BV extract has also shown to possess AChE inhibitory activity and antioxidant potential in vitro [18].

Based on the literature review, there is no study evaluating the effect of BV on cognitive functions in the AD model. With all this information, in the present study, we investigated the effect of BV extract against ICV-STZ induced neuroinflammation and memory impairment in rats through the measurement of AChE activity, TNF-α and IL-1β levels in hippocampus and cerebral cortex, as well as behavioral parameters such as open field test (OFT), novel object recognition test (NORT), passive avoidance test (PAT).

2. RESULTS

2.1. Locomotor movements

According to the open field test results, the number of passes in the STZ and BV groups did not differ from the sham group, suggesting that icv procedure did not cause any major motor impairments in this task (Figure 1). The conventional mode of therapy are often related with drug induced side effect, dose related toxicity and lack of specificity, frequency of dosing and dose dumping, and drug resistance.

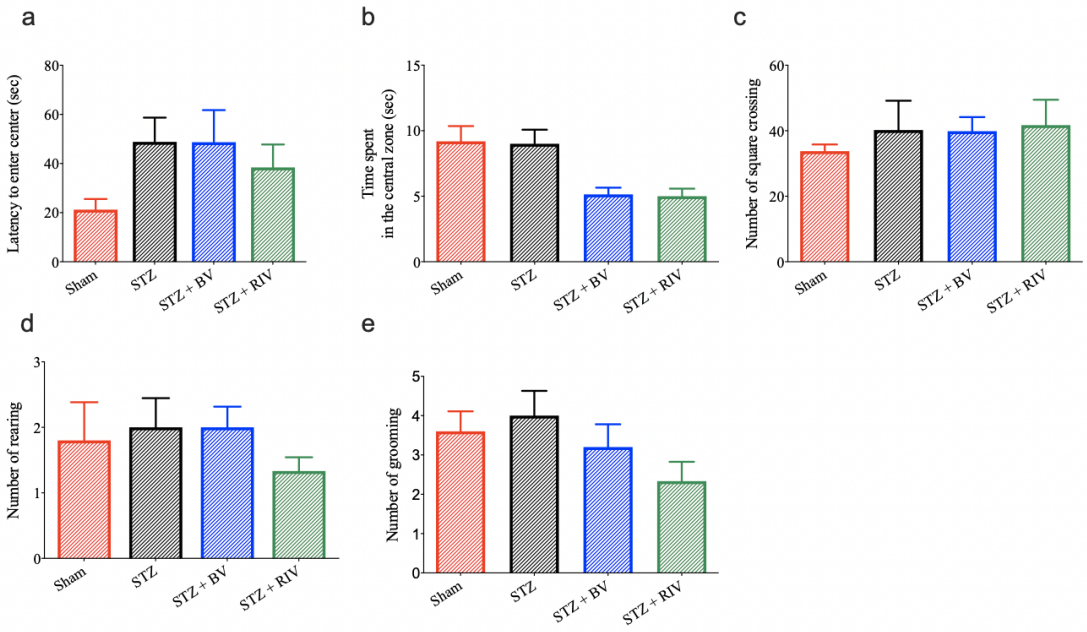

Figure **1.** Effect of *Beta vulgaris* L. var. cicla treatment on the autonomous activities of rats with ICV-STZ in open field test a) Latency to enter the center, b) time spent in the central zoom, c) number of square crosses, d) number of rearing, and e) number of grooming.

2.2. Effect of BV on memory impairments

NORT aims to evaluate and recognize the memory of rats by revealing mild cognitive impairment. The data showed that a significant difference between the exploration time of the familiar and novel object in the sham group, but there is no difference in the STZ induced rats' group (Figure 2a). According to the test results, differences between the groups were shown in the discrimination index and preferential index values. It was obviously viewed that all groups were significantly higher than the STZ group in discrimination index and preferential index (Figure 2 b,c). On the other hand, BV extract treatment group considerably decreased compared to STZ group in discrimination index and preferential index. According to the results, it is seen that BV treatment has a curative effect on short-term memory deficit caused by STZ injection. The latency time in the passive avoidance task was less in the sham group compared to the STZ group. This indicates that STZ-induced impairs memory and learning ability in AD animals. On the other hand, BV treatment improved the STZ induced memory and learning deficits in comparison to STZ group (Figure 2d). The latency time of BV treated group showed no significant differences in memory in compared to RIV treated group rats.

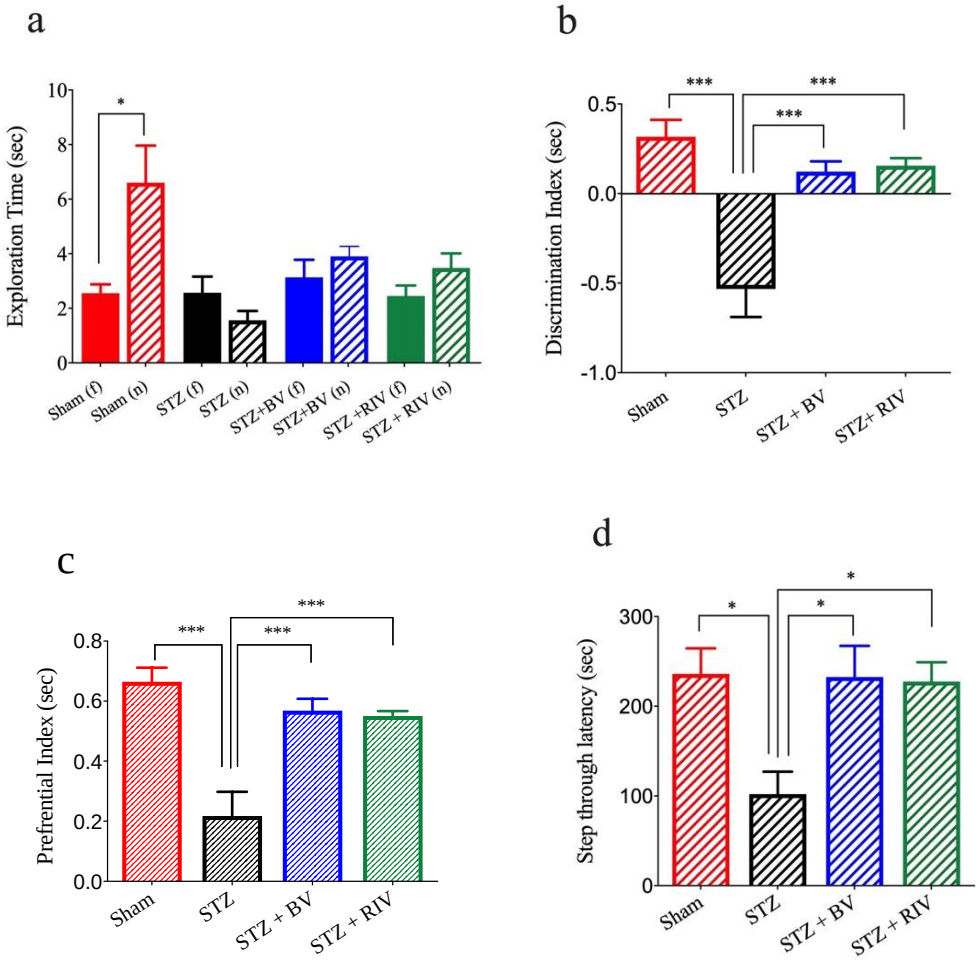

Figure 2. Effect of *Beta vulgaris* L. var. cicla treatment on short-term memory of rats with ICV-STZ. Novel object recognition test: a) Exploration time, b) discrimination index, and c) preferential index. Passive avoidance test (PAT): d) Step-through latency. Significance differences were found at * $p < 0.05$, *** $p < 0.001$.

2.3. Cholinergic activities of BV on hippocampus and cerebral cortex

It is known that increased acetylcholinesterase (AChE) activity disrupts cognitive function and memory by decreasing cholinergic transmission. Figure 3 shows the effect of STZ and BV-treated on the AChE activity in the hippocampus and cerebral cortex. ICV-STZ administration led to increase in AChE activity compared to sham group hippocampal and cerebral cortex parts. BV treatment significantly decreased ($p < 0.01$) AChE activity in the hippocampus and cerebral cortex parts of ICV-STZ injected rats.

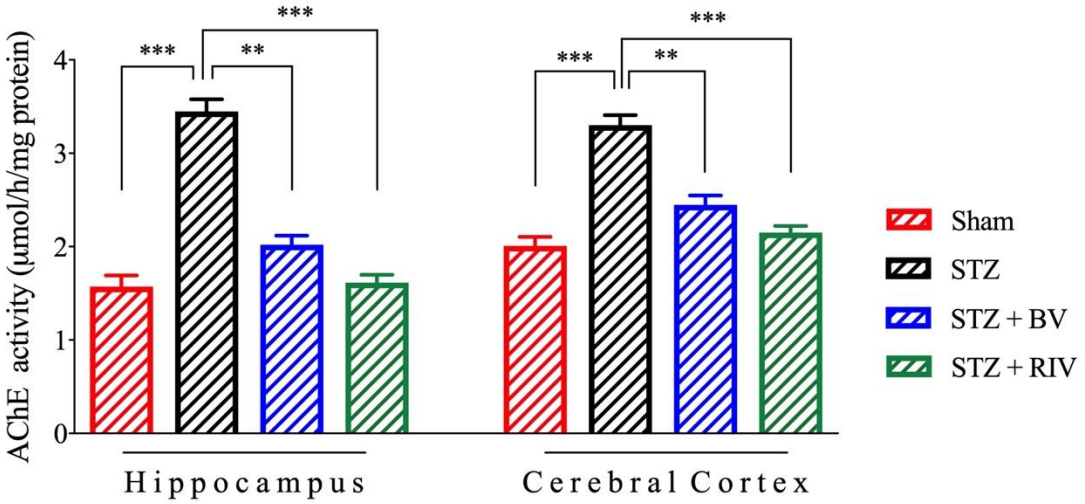

Figure 3. Effect of *Beta vulgaris* L. var. cicla treatment on acetylcholinesterase (AChE) activity in hippocampus and cerebral cortex of rats with ICV-STZ. ** p <0.01, *** p<0.001.

2.4. Effect on BV on TNF-α levels on hippocampus and cerebral cortex

STZ (3 mg/kg) via ICV administration produced significant ($p < 0.001$) elevation in hippocampus and cerebral cortex TNF-α levels as compared to sham group (Figure 4). Treatment with BV apparently attenuated the increased TNF-α levels in hippocampus and cerebral cortex ($p < 0.01$) when compared with STZ group. Besides, brain TNF- α level of RIV group was significantly lower than of BV ($p < 0.001$) in both hippocampus and cerebral cortex.

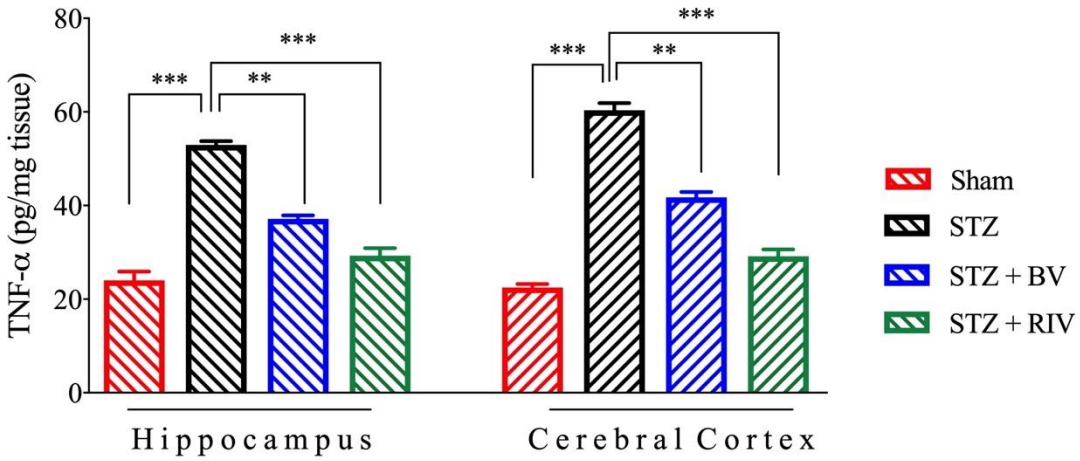

Figure 4. Effect of *Beta vulgaris* L. var. cicla treatment on TNF-α activity in hippocampus and cerebral cortex of rats with ICV-STZ. ** p <0.01, *** p<0.001.

2.5. Effect on BV on IL-1β levels on hippocampus and cerebral cortex

The ICV-STZ group rats exhibited considerable rise in brain IL-1β level in comparison to sham group, this elevation refer to induction of neuro-inflammation. A substantial increase ($p < 0.001$) in IL-1β levels in both hippocampus and cortex tissue was observed in the STZ group compared to the sham group (Figure 5). Administration of BV significantly inhibited the increase of IL-1β levels in the hippocampus and the cerebral cortex of ICV-STZ rats. In both the hippocampus and the cortex, RIV treated group had lower ($p < 0.001$) IL-1β level with respect to BV-treated group.

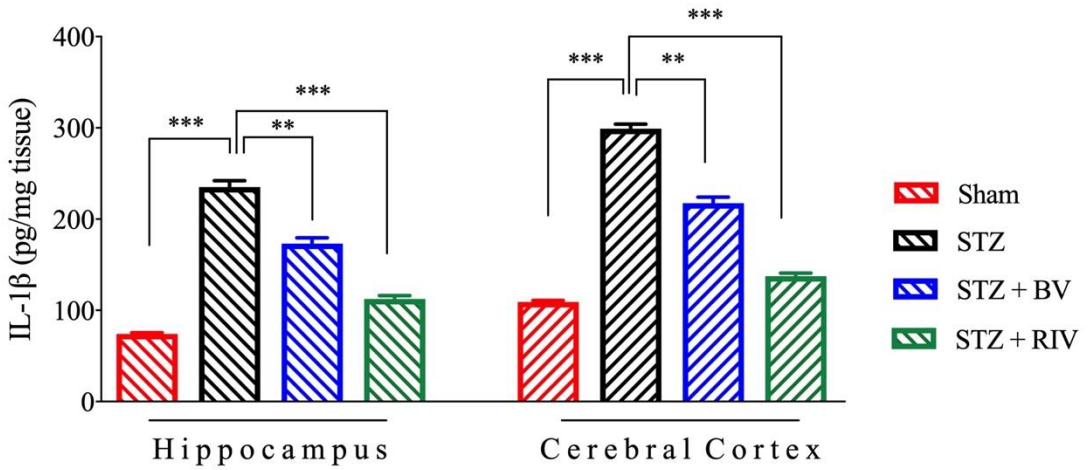

Figure 5. Effect of *Beta vulgaris* L. var. cicla treatment on IL-1β levels in hippocampus of rats with ICV-STZ. ** p <0.01, *** p<0.001.

3. DISCUSSION

The findings of our present study showed that BV treatment provided significant amelioration against STZ-induced cognitive impairment, cholinergic dysfunction and neuroinflammation in the AD. ICV injection of STZ is widely preferred as an animal model in pharmacological studies because it produces behavioral and neuropathological results similar to sporadic AD [19]. Although the mechanism has not been fully elucidated, studies have confirmed that cognitive decline and learning and memory impairments induced by STZ are associated with down-regulation of the insulin pathway, accumulation of β-amyloid and tau proteins, impaired cholinergic transmission, and increased proinflammatory cytokine level [20, 21]. Plant extracts and natural products appear as an alternative treatment option due to their rich content to prevent neurodegenerative diseases such as AD. Although acetylcholinesterase and antioxidant activity of BV extract has been demonstrated in vitro, its effect on cognitive functions and neurochemical parameters has not been clarified before. In this study, we reported for the first time that BV ameliorated cognitive deficits induced by STZ in rats through inhibition of AChE enzyme and attenuating neuroinflammation.

The absence of a significant difference between the groups in locomotor activity according to OFT results indicates that the surgical operation was performed safely. According to the NORT results, the time to be interest with the new object compared to the old object of STZ-induced rats is considerably shorter than that of the sham group. Accordingly, it confirms that STZ causes memory impairment in rats. A noticeable increase in discrimination and preferential index compared to the STZ group after treatment with BV indicates an improvement in short-term memory functions. The PAT is used to evaluate emotional memory in situations accompanied by fear/anxiety through a stimulus [22]. In parallel with the literature, it was observed that rats exposed to STZ exhibited cognitive deficits in the PAT task [23]. STZ-induced decrease in step through latency in PAT was significantly reversed by BV treatment. Our results showed that STZ impairs memory acquisition (short-term memory) in both assessment tasks. After BV treatment, increase in DI and PI in NORT test and decrease in PAT step through latency were interpreted as signs of improvement in STZ-induced cognitive impairment.

As it is well known, one of the main features of AD pathology is a decrease in cholinergic transmission due to increased AChE activity [24]. AChE breaks down acetylcholine, a neurotransmitter closely associated with learning and memory, thereby inhibiting its effects on cognition [25]. Hence, targets to reduce AChE activity have potential role in the treatment of AD. In this study, STZ administration caused a significant increase in AChE activity in the hippocampal and cortical tissues. On the other had treatment with BV extract decreased the AChE enzyme activity at a level like that of the rivastigmine group, the positive control group that given standard drug, in both hippocampus and cortex. Our results were in support of finding of Sacan and Yanardag summarizing the inhibitory effect of BV extract on AChE activity in vitro. According to this report [18], the anticholinesterase and antioxidant activity of the BV extract is closely related to the total phenolic compound content.

Neuroinflammation is an essential innate mechanism that allows the brain to protect itself against infection and injury [10]. However, with the excessive activation of this mechanism, microglia and astrocytes are activated, causing an increase in proinflammatory cytokines such as IL-6, IL-1β and TNF-α [9]. TNF-α released by lymphocytes, fibroblasts, leukocytes and epithelial cells has an effect on many signaling pathways, thereby triggering neuroinflammation by increasing the expression of proinflammatory cytokines such as Il-6 and Il-1β [26]. As a result, the breaking of synapse connections and neuronal deaths in the brain form the basis of the pathology of AD [27]. Previous studies have proven that increased levels of IL-6, IL-1β and TNF-α in the brains of AD patients are associated with weakening of connections between neurons in different parts of the brain and accelerating the formation of amyloid β [28]. Studies focusing on the use of non-steroidal anti-inflammatory agents such as indomethacin, sulindac, and ibuprofen in patients with AD have reported that long-term use of these drugs can potentially reverse the neuroinflammation, however their side effects are limiting factor in their use [29]. There are studies suggesting the potential protective efficacy of cognition by preventing neuroinflammation of the treatment with plants containing saponins and polyphenolic compounds such as flavonoids [30]. As expected, in the present study TNF-α and IL-1β levels increased in the hippocampus and cerebral cortex of rats induced by ICV-STZ, while these cytokines were noticeably reduced in both tissues with BV extract treatment. It is well known that AD-like cognitive dysfunction and behavioral disorders result from overstimulation and uncontrolled of the inflammatory response [31]. Thus, our results suggest that the observed improvement in cognitive abilities of STZ-induced rats may be related to the neuroinflammation-reducing effects of BV extract content.

4. CONCLUSION

In summary, from the current observations, it can be concluded that the cognitive improvement in STZ-infused rats following BV treatment could be due to its anticholinesterase and anti-inflammatory activities and its ability to modulate hippocampal and cerebral cortex neurochemistry. The memory improvement effects of BV can be attributed by its ability to improve cholinergic neurotransmission and decrease proinflammatory cytokines. Nevertheless, further studies need to be carried out to explore the exact pathways responsible for the neuroprotective actions of BV.

5. MATERIALS AND METHODS

5.1. Chemical

Streptozotocin (STZ) and acetylthiocholine iodide (AChI) 5,5′-dithiobis(2- nitrobenzoic acid) (DTNB) were purchased from Sigma- Aldrich, USA. STZ was diluted in citrate buffer (pH 4.4) in a 0.9 % (w/v) saline. Interleukin-1β (IL-1β; Cat No: E2206Ra) and tumor necrosis factor-α (TNF-α; Cat No: E0764Ra) kits were purchased from Bioassay Technology Laboratory (BT Lab) is a brand of Shanghai China. Unless stated, all other chemicals and biochemical reagents of highest analytical grade were used for the study. Solutions of the drug and chemicals were freshly prepared before use.

5.2. Preparation of *Beta vulgaris* L. var. cicla extract

Beta vulgaris L. var. cicla (BV) leaves were collected from Istanbul, Turkey. Plant material was washed with distilled water and dried at room temperature. Briefly, dried chard leaves (100 g) were extracted with the addition of 1000 mL distilled water and boiled for 30 min. Then the extract was filtered, and the filtrate was evaporated under reduced pressure using a rotary evaporator (Bibby RE-100 B, Yamato, CA). The extract was dissolved in distilled water and administered to the rats at o dose of 2 g/kg every day for 21 days via gavage through an intragastric tube [32].

5.3. Animals

Adult male Sprague-Dawley rats (300-350 g) were purchased from Marmara University Experimental Animal Implementation and Research Center. The rats were housed under in regulated rooms with controlled temperature (20-23°C), humidity (40-60%) and light (12 h light/dark regime). A standard rodent pellet diet with tap water was available *ad libitum* to the animals. The permission for the animal experiments was granted by the Animal Experiments Local Ethics Committee at Marmara University (approval number: 29.2019.mar). For the training (handling) phase, one week before the experiment, the animals had a process of getting used to the experimental environment and the researcher.

5.4. Experimental design of study

Rats were anesthetized with 100 mg/kg ketamine and 0.75 mg/kg chlorpromazine via intraperitoneally. Animals placed in a stereotaxic frame and according to the bregma point, coordinates was determined for the injection site: anteroposterior, −0.8 mm from the bregma; lateral, ± 1.5 mm from the sagittal suture; ventral, −3.1 mm from the skull. STZ and treatmet groups were infused with 3 mg/kg STZ (5 µl into the each ventricle;), with with a 28 gauge Hamilton syringe.Sham group animals received the same volume of saline (%0.9; 5 µl/each site) instead of STZ [33].

Animals were divided into four groups: Control (Sham), STZ, STZ+BV and STZ+Riv. Each groups comprised of twelve animals.BV extract was given at doses of 2 g/kg (intragastic, i.g), and Rivastigmin (RIV) at doses of 1.5 mg/kg (i.g). Treatments were continued once daily for a period of 21 days, starting from day 3 after the first dose of STZ administration. The identification of effective dose of BV was based on previous our studies [16].

5.5. Behavioural assesment

5.5.1. Open field test

The spontaneous locomotor activities of the animals were observed to check the effect of the surgical procedure on the study. The square (50x50x30 cm^3) arena with a white surface placed in a moderately lit room was divided into 25 equal squares with 60 cm high walls and black lines. The test was started by placing a rat in the starting corner and allowing it to move around and observing it for 5 minutes. At the end of the experiment, the total time in the inner/outer parts, the number of inner/outer squares entered by the rats, the measurement of the number of lines crossed, the four paws in the inner/outer arenas were measured with all rat entrance frequency parameters. A camera was positioned on the ceiling to monitor the behavior of the rats during the experiment. At the end of each test, the arena was cleaned with 70% ethanol and let to dry for 5 minutes [34].

5.5.2. New object regocnation test

NORT was performed on animals on the 17th day for the evaluation of short-term memory functions [35]. The test was carried out in a semi-dark environment in a black box (50x50x30 cm^3). The lapping process was performed for all animals. While the new object (N1 and N2) and the familiar object (F1 and F2) are of similar height and volume, they have a different shape and appearance. On the first stage, is called the training phase (T1), each animal was placed in the apparatus for 3 minutes at 60 minutes intervals. During these 3 minutes, an animal was allowed to move freely in the apparatus to recognize the same two previously placed objects. After 3 minutes, the animal was placed back in its cage. On the second stage, recognition phase (T2), one of the same two objects was changed, and 60 minutes later, the same animal was retaken to the apparatus. It was allowed to move freely in the apparatus for 3 minutes. Before starting the each test the objects and apparatus were flushed with 70% ethanol. Exploration of the object was defined as the animal's nose being in a zone within 2 cm from the object. In the two stages, the animals' interest in both objects was recorded employing a computer-assisted camera system. The time spent exploring each object, the discrimination index (DI) and prefrential index (PI) were measured [36]. The results of the test were expressed as Eq. 1 and Eq. 2.

$$Discrimination\ Index = (tN1 - tF1)/(tN1 + tF1) \qquad \text{(Eq. 1)}$$

$$Preferential\ Index = tN1/\ (tN1 + tF1) \qquad \text{(Eq.2)}$$

5.5.3. Passive avoidance test

The passive avoidance test (PAT) was performed to evaluate working memory functions as previously described by Kameyama et al [37]. This test was performed in apparatus consisted of two compartments, one bright and one dark, each of which has 20x25x30 cm^3 dimensions separated by a controlled sliding door. The test performed to consecutive days consists of two stages. In stage one, rats were put one by one in the bright room and after 10 seconds the door was opened in the experiment and the rats were pursued to passing the dark compartment. 24 hours after this winning part, the other stage called as the avoidance experiment was carried out. In this part, rats were put in the bright room again. The latency of the time for rats from the bright room to the dark room was recorded. If the rats did not cross for 300 seconds, the transition time was accepted as 300 seconds [38].

5.6. Collection of brain samples

After the behavioral assessment on 21th day, animals were sacrificed and brain tissue samples were immediately removed and excised for further acetylcholinestrease (AChE) activity and neuroinflammatory markers. After isolating the whole brains, the hippocampus and cerebral cortex were dissected on a cold plate. The tissue samples were stored at − 80 °C until further analysis. The treatment procedure is shown in Figure 6.

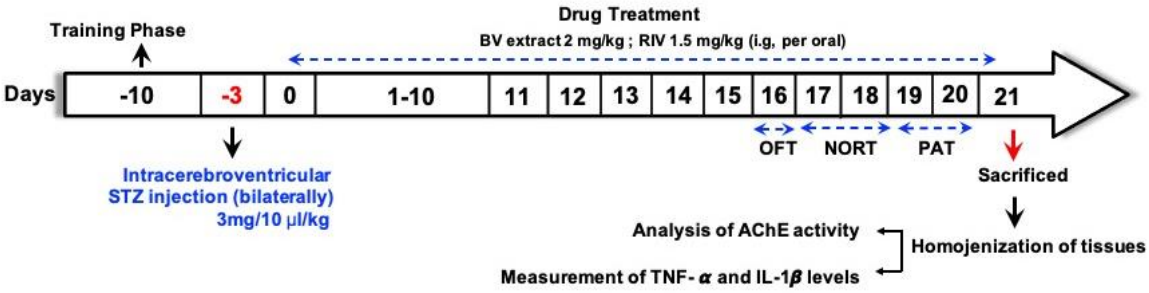

Figure 6. Experiment procedure and treatment schedule. STZ, streptoztocin; BV, *Beta vulgaris* L. var. cicla; RIV, Rivastigmine; OFT, open field test; NORT, novel object regocnation test; PAT, passive avoidance test; AChE, Acetylcholinesterase.

5.7. Analysis of acetylcholenesterase (AChE) activity

The method of Ellman et al. was used to assay acetylcholinesterase (AChE) activities [39]. The hippocampus and cerebral cortex tissues were homogenized with phosphate buffer (pH 7.4). 15 µL of the brain homogenate was added to 100 µL of 100 mM phosphate buffer (pH 7.4) and Ellman reactive DTNB (100 µL, 1.7 mM) and 1.4 mM acetylthiocholine iodide. AChE activity was monitored at 412 nm. Enzyme activity was calculated and expressed as µmol AChE per min per mg of protein.

5.8. Analysis of proinflammatory cytokines (TNF-α and IL-1β) levels on hippocampus and cerebral cortex

The hippocampal and cortical tissues were homogenized with IKA brand Ultra-Turrax T25 (USA) homogenizer in cold PBS solution to obtain a 10% (w/v) homogenate solution [40]. After the homogenate samples (10% w/v) were then centrifuged (Remi cold centrifuge) at 10,000g for 15min and supernatant was collected for further estimation of following biochemical parameters. Biochemical parameters analyzed using ELISA kits provided from Bioassay Technology Laboratory (BT Lab) is a brand of Shanghai China. All procedures were performed according to the manufacturer's instructions. Concentrations of proinflammatory cytokines were calculated from the standard curves.

5.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, 230 USA). All data are expressed as the mean ± standart error mean (SEM). The results of the comparisons of total time of exploration during T1 and T2 on familiar and novel object were analyzed by using two-way ANOVA followed by Bonferroni's post hoc test. The results of AChE activity and biochemical datas were analyzed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was accepted as $p < 0.05$.

Author contributions: Concept – B.E., G.Ş.; Design – B.E., G.Ş.; Supervision – B.E., R.G., G.Ş.; Resources – B.E., O.S., G.Ş.; Materials – B.E., R.Y., G.Ş.; Data Collection and/or Processing B.E., F.T., G.Ş.; Analysis and/or Interpretation – B.E., G.Ş.; Literature Search- B.E., F.T., G.Ş.; Writing – B.E., G.Ş.; Critical Reviews – B.E., F.T., R.G., R.Y, O.S., G.Ş.

Conflict of interest statement: The authors declare that they have no competing interests.

Ethics committee approval: All experimental protocols were performed according to the Marmara University Animal Experiments Local Ethics Committee, İstanbul- Turkey (Protocol number: 29.2019.mar) on April 1, 2019.

REFERENCES

[1] Perl DP. Neuropathology of Alzheimer's disease. Mt Sinai J Med. 2010; 77: 32-42. [CrossRef]

[2] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002; 297: 353-356. [CrossRef]

[3] Weinreb O, Amit T, Bar-Am O, Youdim MB. Ladostigil: a novel multimodal neuroprotective drug with cholinesterase and brain-selective monoamine oxidase inhibitory activities for Alzheimer's disease treatment. Curr Drug Targets. 2012; 13: 483-494. [CrossRef]

[4] Liu C, Wu Y, Zha S, Liu M, Wang Y, Yang G, Ma K, Fei Y, Zhang Y, Hu X, Yang W, Qian Y. Treatment effects of tanshinone IIA against intracerebroventricular streptozotocin induced memory deficits in mice. Brain Res. 2016; 1631: 137-146. [CrossRef]

[5] Kadıoğlu Yaman B, Çevik Ö, Yalman K, Ertaş B, Şen A, Şener G. Myrtus communis subsp. communis improved cognitive functions in ovariectomized diabetic rats. Gene. 2020; 744: 144616. [CrossRef]

[6] Cevikelli-Yakut ZA, Ertas B, Sen A, Koyuncuoglu T, Yegen BC, Sener G. Myrtus communis improves cognitive impairment in renovascular hypertensive rats. J Physiol Pharmacol. 2020; 71. [CrossRef]

[7] Mohamed EA, Ahmed HI Zaky HS, Badr AM. Sesame oil mitigates memory impairment, oxidative stress, and neurodegeneration in a rat model of Alzheimer's disease. A pivotal role of NF-κB/p38MAPK/BDNF/PPAR-γ pathways. J Ethnopharmacol. 2021; 267: 113468. [CrossRef]

[8] Yan Z, Feng J. Alzheimer's disease: interactions between cholinergic functions and beta-amyloid. Curr Alzheimer Res. 2004; 1: 241-248. [CrossRef]

[9] Yang W, Liu Y, Xu QQ, Xian YF, Lin ZX. Sulforaphene Ameliorates Neuroinflammation and Hyperphosphorylated Tau Protein via Regulating the PI3K/Akt/GSK-3β Pathway in Experimental Models of Alzheimer's Disease. Oxid Med Cell Longev. 2020; 2020: 4754195. [CrossRef]

[10] Zhang F, Jiang L. Neuroinflammation in Alzheimer's disease. Neuropsychiatr Dis Treat. 2015; 11: 243-256. [CrossRef]

[11] Reeta KH, Singh D, Gupta YK. Chronic treatment with taurine after intracerebroventricular streptozotocin injection improves cognitive dysfunction in rats by modulating oxidative stress, cholinergic functions and neuroinflammation. Neurochem Int. 2017; 108: 146-156. [CrossRef]

[12] Kandimalla R, Thirumala V, Reddy PH. Is Alzheimer's disease a Type 3 Diabetes? A critical appraisal. Biochim Biophys Acta Mol Basis Dis. 2017; 1863: 1078-1089. [CrossRef]

[13] Paidi RK, Nthenge-Ngumbau DN, Singh R, Kankanala T, Mehta H, Mohanakumar KP. Mitochondrial Deficits Accompany Cognitive Decline Following Single Bilateral Intracerebroventricular Streptozotocin. Curr Alzheimer Res. 2015; 12: 785-795. [CrossRef]

[14] Grutzendler J, Morris JC. Cholinesterase inhibitors for Alzheimer's disease. Drugs. 2001; 61: 41-52. [CrossRef]

[15] Hashem AN, Soliman MS, Hamed MA, Swilam NF, Lindequist U, Nawwar MA. Beta vulgaris subspecies cicla var. flavescens (Swiss chard): flavonoids, hepatoprotective and hypolipidemic activities. Pharmazie. 2016; 71: 227-232. [CrossRef]

[16] Bolkent S, Yanardağ R, Tabakoğlu-Oğuz A, Ozsoy-Saçan O. Effects of chard (Beta vulgaris L. var. Cicla) extract on pancreatic B cells in streptozotocin-diabetic rats: a morphological and biochemical study. J Ethnopharmacol. 2000; 73: 251-259. [CrossRef]

[17] Mohammed HS, Abdel-Aziz MM, Abu-Baker MS, Saad AM, Mohamed MA, Ghareeb MA. Antibacterial and Potential Antidiabetic Activities of Flavone C-glycosides Isolated from Beta vulgaris Subspecies cicla L. var. Flavescens (Amaranthaceae) Cultivated in Egypt. Curr Pharm Biotechnol. 2019; 20: 595-604. [CrossRef]

[18] Sacan O, Yanardag R. Antioxidant and antiacetylcholinesterase activities of chard (Beta vulgaris L. var. cicla). Food Chem Toxicol. 2010; 48: 1275-1280 [CrossRef]

[19] Choi DH, Kwon IS, Koo JH, Jang YC, Kang EB, Byun JE, Um HS, Park HS, Yeom DC, Cho IH, Cho JY. The effect of treadmill exercise on inflammatory responses in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. J Exerc Nutrition Biochem. 2014; 18: 225-233. [CrossRef]

[20] Arora R, Deshmukh R. Correction to: Embelin Attenuates Intracerebroventricular Streptozotocin-Induced Behavioral, Biochemical, and Neurochemical Abnormalities in Rats. Mol Neurobiol. 2020; 57: 3991. [CrossRef]

[21] Halawany AME, Sayed NSE, Abdallah HM, Dine RSE. Protective effects of gingerol on streptozotocin-induced sporadic Alzheimer's disease: emphasis on inhibition of β-amyloid, COX-2, alpha-, beta -secretases and APH1a. Sci Rep. 2017; 7: 2902. [CrossRef]

[22] Gerzson MFB, Bona NP, Soares MSP, Teixeira FC, Rahmeier FL, Carvalho FB, Da Cruz Fernandes M, Onzi G, Lenz G, Gonçales RA, Spanevello RM, Stefanello FM. Tannic Acid Ameliorates STZ-Induced Alzheimer's Disease-Like Impairment of Memory, Neuroinflammation, Neuronal Death and Modulates Akt Expression. Neurotox Res. 2020; 37: 1009-1017. [CrossRef]

[23] Adeli S, Zahmatkesh M, Ansari Dezfouli M. Simvastatin Attenuates Hippocampal MMP-9 Expression in the Streptozotocin-Induced Cognitive Impairment. Iran Biomed J. 2019; 23: 262-271. [CrossRef]

[24] Kim W, Yoo DY, Jung HY, Kim JW, Hahn KR, Kwon HJ, Yoo M, Lee S, Nam SM, Yoon YS, Kim DW, Hwang IK. Leaf extracts from Dendropanax morbifera Léveille mitigate mercury-induced reduction of spatial memory, as well as cell proliferation, and neuroblast differentiation in rat dentate gyrus. BMC Complement Altern Med. 2019; 19: 94. [CrossRef]

[25] Kamdi SP, Raval A, Nakhate KT. Phloridzin attenuates lipopolysaccharide-induced cognitive impairment via antioxidant, anti-inflammatory and neuromodulatory activities. Cytokine. 2021; 139: 155408. [CrossRef]

[26] Wang WY, Tan MS, Yu JT, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Ann Transl Med. 2015; 3: 136. [CrossRef]

[27] Rai S, Kamat PK, Nath C, Shukla R. A study on neuroinflammation and NMDA receptor function in STZ (ICV) induced memory impaired rats. J Neuroimmunol. 2013; 254: 1-9. [CrossRef]

[28] Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finsen B, Brown GC, Verkhratsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Petzold GC, Town T, Morgan D, Shinohara ML, Perry VH, Holmes C, Bazan NG, Brooks DJ, Hunot S, Joseph B, Deigendesch N, Garaschuk O, Boddeke E, Dinarello CA, Breitner JC, Cole GM, Golenbock DT, Kummer MP. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 2015; 14: 388-405. [CrossRef]

[29] Khan MA, Alam Q, Haque A, Ashafaq M, Khan MJ, Ashraf GM, Ahmad M. Current Progress on Peroxisome Proliferator-activated Receptor Gamma Agonist as an Emerging Therapeutic Approach for the Treatment of Alzheimer's Disease: An Update. Curr Neuropharmacol. 2019; 17: 232-246. [CrossRef]

[30] Medrano-Jiménez E, Jiménez-Ferrer Carrillo I, Pedraza-Escalona M, Ramírez-Serrano CE, Álvarez-Arellano L, Cortés-Mendoza J, Herrera-Ruiz M, Jiménez-Ferrer E, Zamilpa A, Tortoriello J, Pedraza-Alva G, Pérez-Martínez L. Malva parviflora extract ameliorates the deleterious effects of a high fat diet on the cognitive deficit in a mouse model of Alzheimer's disease by restoring microglial function via a PPAR-γ-dependent mechanism. J Neuroinflammation. 2019; 16: 143. [CrossRef]

[31] Wang D, Wang C, Liu L, Li S. Protective effects of evodiamine in experimental paradigm of Alzheimer's disease. Cogn Neurodyn. 2018; 12: 303-313. [CrossRef]

[32] Oztay F, Sacan O, Kayalar O, Bolkent S, Ipci Y, Kabasakal L, Sener G, Yanardag R. Chard (Beta vulgaris var. cicla) extract improved hyperglycemia-induced oxidative stress and surfactant-associated protein alterations in rat lungs. Pharm Biol. 2015; 53: 1639-1646. [CrossRef]

[33] Paxinos G, Watson CR. The rat brain in stereotaxic coordinates. Academic Press. 1998; 1: 129-149. [CrossRef]

[34] Cunha JM, Masur J. Evaluation of psychotropic drugs with a modified open field test. Pharmacology. 1978; 16: 259-267. [CrossRef]

[35] Özbeyli D YG, Ertas B, Şen a, Şakarcan S, Şener G Myrtus communis extract ameliorates high-fat diet induced brain damage and cognitive function. J.Res.Pharm. 2020; 24: 865-873. [CrossRef]

[36] Aykac A, Ozbeyli D, Uncu M, Ertaş B, Kılınc O, Şen A, Orun O, Sener G. Evaluation of the protective effect of Myrtus communis in scopolamine-induced Alzheimer model through cholinergic receptors. Gene. 2019; 689: 194-201. [CrossRef]

[37] Kameyama T, Nabeshima T, Kozawa T. Step-down-type passive avoidance- and escape-learning method. Suitability for experimental amnesia models. J Pharmacol Methods. 1986; 16: 39-52. [CrossRef]

[38] Reeta KH, Singh D, Gupta YK. Edaravone attenuates intracerebroventricular streptozotocin-induced cognitive impairment in rats. Eur J Neurosci. 2017; 45: 987-997. [CrossRef]

[39] Ellman GL, Courtney KD, Andres V, Jr., Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7: 88-95. [CrossRef]

[40] Lu Y, Dong Y, Tucker D, Wang R, Ahmed ME, Brann D, Zhang Q. Treadmill Exercise Exerts Neuroprotection and Regulates Microglial Polarization and Oxidative Stress in a Streptozotocin-Induced Rat Model of Sporadic Alzheimer's Disease. J Alzheimers Dis. 2017; 56: 1469-1484. [CrossRef]