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Evaluating the effects of basil (*Ocimum basilicum*) in the experimental model of Alzheimer's disease in rats

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Alzheimer is a destructive neurodegenerative disease related to elderly age of people and it is described by memory decline, loss of cognitive and psychiatric characters. The *Ocimum* species has been used for anti-ulcerogenic, anti-inflammatory and neuroprotective activities. Therefore, this study was conducted to assess the possible role of basil (Dino cultivar) in rats against Alzheimer disease (AD). Dino cultivar was selected among the 16 basil genotypes and 4 cultivars growing under field condition in 2019 vegetation period. Some quality properties such as essential oil and its compositons and extract yield of the Dino cultivar were determined. Also, biochemical parameters and immunohistochemical findings and behavioral test were obtained in rats against AD.

Dino had high essential oil content (1.28%), five major essential oil compositions (*p*-Allyl-anisole, linalool, trans-caryophyllene, methyl eugenol and beta bergamontene) and extract yield. The extract of the Dino cultivar was used for the biochemical parameters of the Alzheimers as total antioxidant status (TAS: 0.21-0.82 mmol/L), total oxidant status (TOS: 5.7-15.23 µmol/L), catalase (CAT: 2.17-177.31 U/L), oxidative stress index (OSI: 1.056-3.818), malondialdehyde (MDA: 0.365-2.022 mmol/L), superoxide dismutase (SOD: 194-207 U/mL) and Glutathione peroxidase1 (GPX1: 10.82-18.22 ng/mL) in experimental rats. Also, the effect of the extract was used to determine immunohistochemical findings and behavirol test results.

As a result of the study, the extract of the Dino cultivar had positive effect on AD depending on the examined properties. It can be suggested that basil extract can be used for the treatment of AD.

Keywords: Alzheimer, Basil, Biochemical paramaters, Behavioral test

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Alzheimer's disease (AD) is the most common form of dementia in the elderly, estimated to affect more than 50 million worldwide, and with almost half of individuals aged over 75 years[1]. The most important defining symptoms of AD are memory loss and cognitive dysfunction. In fact, these neuropathological manifestations begin long before the obvious cognitive symptoms.

This cognitive dysfunction is known to include accumulation of amyloid-beta (Aβ) peptide as extracellular plaques and hyperphosphorylated tau as intracellular neurofibrillary tangles (NFTs), typically identified on postmortem biopsy and used for definitive AD diagnosis. Because of the severity and increasing prevalence of the disease in the population, it is urgent that better treatments be developed[2]. Food and Drug Adiministration has developed two drugs for AD. However, the results are not very promising. So, alternative drugs can be used in AD. Natural therapy containing herbs has long been used to treat memory disorders such as dementia, amnesia and AD[3].

Ocimum basilicum L. (sweet basil) is one of the most important spices belonging to the Labiatae family. Basil is most widely used due to its high economical value, popularity and widely cultivated for essential oil production in India, Turkey, Iran, Japan and Chine[4]. It has been used in traditional medicine as a folklore cure for comprehensive treatments in the world.

Some investigations have shown its various protective effects including radiation protective efficacy, preventive potential against some chemicals, anti-inflammatory effect, stimulant agent in central nervous system, bactericidal activity, modulatory effect on glutathione and improvement in cognitional task, ulcer protective[5-7]. Also, pharmacological studies have

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demonstrated that the basil extracts have potent antioxidant activities, by preventing lipid peroxidation[8], but also beneficial effects in cognitive disorders like AD, actively enhancing the memory of experimental animal models[9]. Anti-oxidants are effective for AD because they aid in reducing the free radicals that damage the brain cells[10]. Basil safety in animal and human models has been confirmed[11].

Since basil genotypes are foreign pollinated, they have very different chemotypes in terms of chemical content. In these studies, 4 different chemotypes were revealed; methyl chavicol (Estragole, *p*-Allyl-Anisole)-rich, linalool-rich, methyl eugenol-rich, and methyl cinnamate-rich[12].

For this reason, we have been working on the yield and quality characteristics of many basil genotypes for many years. As a result of these studies, genotypes with high yield and quality values were determined. In this study, the most efficient and high quality variety was used in order to ensure continuity and to have a high economic return.

Basil genotypes showed wide variability in yield and quality properties. For example, fresh herb yield of 28 Ethiopian basil genotypes per plant harvest varied from 175.63 g to 718.3 g[13]. In addition, Yaldiz and Camlica[14] reported that essential oil content ranged from 0.04 to 1.71% which is relatively similar with Egata *et al.*[13]. So, basil genotypes were evaluated for quantitative and quality properties and dino cultivar were superior in terms of desired properties among the other basil genotypes and cultivars.

The sweet basil extract has been reported to have a protective effect on neurons of the brain in rats exposed to Alzheimer's disease. However, no studies have comprehensively presented the effect of basil extract on AD by biochemical parameters, immunohistochemical findings and behavirol test results in detail. This study can be seen as first comphrensive report to evaluate basil extract on Alzheimer. Also, the used cultivar was selected among the 20 genotypes depending on the agronomical technologies as morphological, yield and yield components, and essential oil content and chemical composition profile of essential oil from quality properties were determined.

The study had three main aims; i. evaluating the chemical properties of used basil cultivar in terms of essential oil and compositions, ii. reveal the values of behavioral test results with TAS, MDA SOD, CAT, TOS, OSI and GPX results, iii. determining the histological assessments against the AD.

Materials & Methods

Growth conditions and treatments

The seeds of the different origin 16 basil genotypes were obtained from the United States Department of Agriculture (USDA) with seeds of four commercial basil cultivars (Dino, Midnight, Moonlight, and Large Sweet) were used in the year 2019.

The study was conducted at the Bolu Abant İzzet Baysal University, Research and Application area of the Agriculture Faculty (40°44’44’’N, 31°37’45’’E). Seeds were sown (15 April 2019) according to randomized complete block design having three replicates. The experimental plot was established with five rows, a distance of 0.3 m between each row and 0.2 m between each plant, and the plot size was set up 5.6 m^2.

The climatic values were recorded throughout the vegetation period (April to September), and the means were found between 8.7-21.8°C of temperature, between 0-142.6 kg/m^2 of precipitation and between 56.1-76.7% relative humidity. The experimental area soil properties were clay, 7.56 value of pH, 3.71% of organic matter content, 0.05 kg/da phosphorus content, 108.31 kg/da potassium ratio and 0.0383% salt.

In experimental year, 60 kg/ha diammonium phosphate (DAP) and 20 kg/ha ammonium sulfate (AS) were applied with sowing. Totally, 40 kg/ha AS was applied to basil plants into two splits in July and August. The harvest of the plants was performed at the beginning of flowering by hand, and fresh herb was put into drying oven at 35°C, and the dry herb weight was measured.

Preparation of doses

The suitable dose was selected on the basis of the extract dry weight, and 0.9% sodium chloride was used as solvent reported by Sarahroodi *et al.*[15]. The obtained extracts were stored at -20°C until the beginning of the experiment.

Isolation of essential oil

Isolation of the essential oil analysis was conducted according to TS 8882 method reported by Yaldiz & Camlica[14].

Gas chromatography-mass spectrometry/flame ionization detection (GC-MS/FID)

GC-MS/FID was used for the essential oil compositions and essential oil components of the samples were determined according to the methods of Yaldiz & Camlica[14].

Biochemical measurements

Biochemical measurements were determined as TAS, TOS, MDA, SOD, OSI, CAT and GPX1. The analysis methods for these measurements were conducted as reported by previous studies as follow:

TAS and TOS analysis were measured by an automatic method as described by Erel[16] and Erel[17], respectively.

The OSI was determined by using TAS and TOS values. So, to determine the OSI value, the TAS (mmol) and the TOS (μmol) values were cross-converted and the OSI value was expressed as percentage reported by Erel[17] and. Yumru *et al.*[18] by following formula;

OSI (arbitrary unit) =TOS (μmol H_2O_2 equivalent/L) / TAC (μmol Trolox equivalent/L).

The tissue MDA level, SOD and CAT values were determined by a method reported by Yurtdas *et al.*[19] and The GPX1 analysis was conducted according to Anonymous[20].

Animals and experimental design

The Experimental Animal Center of the Abant İzzet Baysal University contributed a total of 32 mature male Wistar rats (250-300 g) for this investigation.

The rats were divided into four groups, each containing eight individuals, as follows: Group 1 is the control group that received distilled water; Group 2 is the Alzheimer's disease group that received Aβ1-42 (Amyloid-β1-42) solution incubated in distilled water at a rate of 0.5 μL/min; Group 3 is the extract group that received basil (400 mg/kg) orally for 60 days at a rate of 1 mL; and Group 4 is the Alzheimer+basil group that received basil extract during 60 days and then received Aβ1-42 solution incubated in distilled water at a rate of 1 μL/min at the end of the 60th day.

Preparation of Aβ1-42 induced rat model of Alzheimer

Synthetic oligomeric amyloid β1-42 solution was prepared as previously described by Brouillette *et al.*[21].

Behavioral tests

Behavioral tests are used to assess memory and cognitive function. The screening test was Morris Water Maze (MWM)[21].

Histological assessments

The histological assessments of samples were carried out as reported[22].

Statistical analysis

Data analysis was performed by JMP statistical software. One-way ANOVA was used and followed by the *Student-t* test was used to compare the mean of the data at $p<0.05$ level.

Results

Essential oil yield and chemical profile of essential oil

The essential oil compositions of the dino cultivar showed large variation from 0.01% to 13.30% (Table 1). In study, Dino cultivar had high essential oil (1.28%). In total, 49 compounds representing 92.28% of the dino cultivar essential oil was identified (Table 1).

Major components of this oil include *p*-allyl anisole (estragole, 13.30%), linalool (10.32%) and trans-caryophyllene (6.09%), all of which are classified as aromatic oxygenated monoterpenes. In addition, methyl eugenol, another oxygenated aromatic monoterpene, accounts for 4.52%, while α-cadinene, a sesquiterpene hydrocarbon, accounts for 9.19% (Table 1).

Biochemical parameters

TAS (mmol/L), TOS (μmol/L), OSI, SOD (U/mL), MDA (nmol/L), CAT (U/L) values were determined for all groups. Significant differences were not found among the experimental groups for the examined properties ($p<0.05$). As can be seen in Figure 1, TAS values varied between 0.21-0.82 mmol/L and MDA values varied between 0.365-2.022 mmol/L. The highest TAS and MDA values were obtained from Alzhemier group, while the lowest TAS and MDA values were obtained from Alzhemier+basil group. In addition, SOD values varied between 194-207 U/mL and CAT values varied between 2.17-177.31 U/L. The highest SOD and CAT values were obtained from Alzhemier group, while the lowest SOD and CAT values were obtained from Alzhemier group Alzhemier+basil group (Fig. 1). Likewise, TOS values varied between 5.7-15.23 μmol/L and OSI values varied between 1.056-3.818. The highest TOS and OSI values were obtained from Alzhemier group, while the lowest TOS and OSI values were obtained from Alzhemier and Alzhemier+basil group (Fig. 1). GPX values changed between 10.82-18.22 ng/mL and the highest value was found from Alzheimer group. The lowest value was found from basil group and followed by control group.

As a result, the highest values were found from Alzheimer group and the lowest values were found from Alzheimer+basil group. For this context, an extract of basil might be useful as a preventing agent for AD.

Table 1 — Essential oil components of the Dino cultivar

No	EOC	RT	Value (%)	No	EOC	RT	Value (%)
1	α-Pinen	15.52	0.03	26	methyl eugenol	37.81	3.21
2	Mycrene	15.93	0.06	27	*trans*-caryophyllene	38.1	6.09
3	Limonen	18.00	0.08	28	α-Humulene	39.08	1.01
4	Eucalyptol	18.78	1.11	29	epi-bicyclosesquiphellanderen	39.35	0.99
5	ɣ-Terpinen	19.42	0.04	30	ɣ-Muurolene	39.79	0.63
6	α-Terpinolen	20.90	0.07	31	Germacrene-D	40.33	4.47
7	*trans*-Sabinene Hydrate	22.04	0.12	32	α-selinene	40.46	1.03
8	Linalol	23.71	10.32	33	Guaiene /cadinene	40.77	1.82
9	Champher	26.59	0.23	34	ɣ-Elemene	40.91	0.81
10	4-Terpineol	27.23	0.29	35	ɣ-Cadiene	41.24	4.63
11	*p*-Allyl-ananisole	28.24	13.30	36	α-himacholene	41.71	3.67
12	α-Terpineol	28.47	3.83	37	Calamenene	41.93	0.97
13	farnesol	29.96	0.08	38	Cubenol	43.4	0.31
14	*z*-citral	31.18	1.11	39	nerdolidol	43.85	0.25
15	Borrnyl acetate	32.07	0.72	40	Spathulenol	45.67	1.15
16	E-citral	32.54	0.31	41	caryophyllene oxide	46.14	0.88
17	α-cubebene	33.50	0.23	42	Torryol	46.49	2.21
18	chavicol	34.43	0.91	43	Calarene epoxide	47.22	0.28
19	α-copaene	34.90	0.63	44	α-cadinene	47.69	9.19
20	Aromadenderene	35.50	0.30	45	α-Cadinol	48.73	1.50
21	D-germacrene	35.72	0.64	46	α-Eudesmol	49.12	0.71
22	Beta Elemene	35.92	1.79	47	widdrol	49.6	0.32
23	Eugenol	36.73	4.52	48	Palmitic acid	53.47	0.38
24	α-Gurjunene	37.12	0.11	49	Mystric acid	56.01	0.01
25	Beta Bergamontene	37.46	4.93				
Total (%)					92.28		

EOC: Essential oil components, RT: Retention time

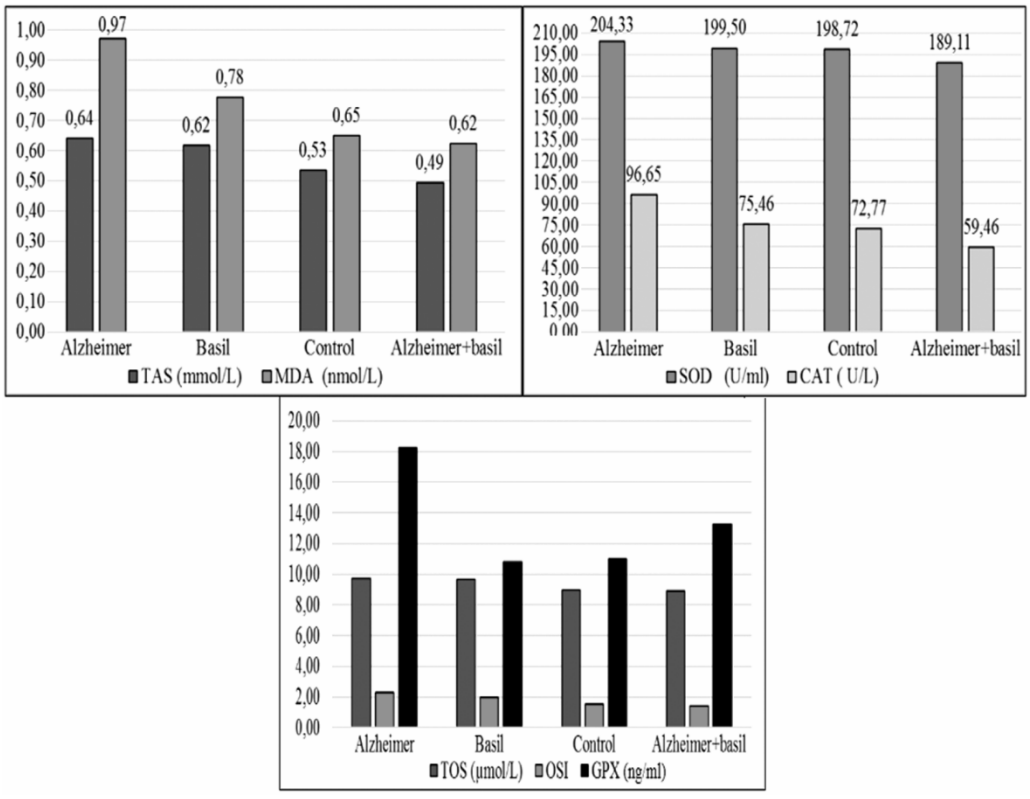

Fig. 1 — Values of TAS, MDA SOD, CAT, TOS, OSI and GPX results

Pathology results

The effect of Dino cultivar against Alzheimer's disease was tested in different groups. Pathology results were noted as absent (O), mild (1), moderate (2) and severe (3) in eight samples of four groups. Neuronal degeneration (ND) was found in at least two samples in all groups. Control group contained 75% absent, and basil and Alzheimer+Alzheimer basil groups had only one moderate in each one. Pyramidal nerve cell loss (PNL) was found as absent in all samples of control and basil group, and it was found only two samples as mild in Alzheimer+basil group. However, Alzheimer group was found as moderate in all samples in terms of PNL.

When compared the cytoplasmic vacuolization (CV) among the samples and groups, generally, the cytoplasmic vacuolization was observed as absent except one sample in control group, it was found as mild in basil group except one sample and as mild or moderate in Alzheimer+basil application. Alzheimer group had the CV more than moderate (5 samples) and severe (3 samples).

Neuronal shrinkage (NS) showed differences compared the other pathological results. It was found as absent except two samples in basil group and except four samples in control group. NS was observed more than 60% as mild and 6 samples were found as moderate in Alzheimer group. Nuclear hyperchromasia (NH) was found only 6 samples as absent in control group and it was found in 4 samples in control, 6 samples in basil group, 7 samples in Alzheimer+basil group as mid. In addition, moderate values were found in two samples, one samples and 5 samples in basil, Alzheimer+basil and Alzheimer groups, respectively. Control group showed differences compared to other groups in terms of congo red amyloid (CA). Because, CA had absent values in control group and basil group (except one samples). Five samples were recorded as mid and 3 samples were found as absent in alzheimer+basil groups. Also, one sample (mild), 5 samples and two samples and were recorded as mild, moderate and severe in Alzheimer groups, respectively.

It can be said that the effect of Alzheimer's disease decreased with the application of the extract of the Dino cultivar compared to Alzheimer group. Severe values were found only alzheimer group, also 3 samples and 4 samples were found as moderate in basil and Alzheimer+basil groups, respectively.

Behavioral test results

The Morris Water Maze test was conducted to determine the spatial and non-spatial memory and learning ability of rats respectively. This test was used to reveal the significant influence on the results. Generally, statistically significant differences were not found in behavioural tests except mean speed. However, a clear tendency was seen in the behavioral tests. The mean of the values changed between 11.60-13.74 min for duration, between 1.61-1.98 m for distance and between 0.13-0.17 m/min for mean speed (Table 2). Time mobile and immobile ranged from 10.62 to12.09 min and 0.46 to 1.83 min, respectively. Line crossing values changed 2.26-2.93 m and path efficiency ratio ranged from 0.60 to 0.63. The highest duration, distance, mean speed and time mobile were found from Alzheimer application. The lowest values on the behavioural test were observed in control application for duration and time mobile and in basil application for distance and mean speed. The highest values were found from Alzheimer and control applications for time immobile, and the highest line crossings and path efficiency values were observed from Alzheimer applications. The lowest time mobile, line crossing and path efficiency ratio values obtained from Alzheimer+basil (4.46 min), basil (2.26 m) and Alzheimer and control applications (0.60), respectively.

Immunohistochemical findings in Alzheimer model

Aβ and tau protein expression in hippocampus tissues; among the adult control, Alzheimer's, basil, and basil-alzheimer's rat groups were compared immunohistochemically. As shown in Figure 2.1, control groups were examined at all three magnifications (HEx40, HEx100, HEx400) in

Table 2 — Values of behavioral test results

Application	Duration (min)	Distance (m)	Mean speed (m/min)	Time mobile (min)	Time immobile (min)	Line crossings (m)	Path efficiency ratio
Alzheimer	13.74^{ns}	1.98^{ns}	0.17a	12.09^{ns}	1.67^{ns}	2.59^{ns}	0.62^{ns}
Alzheimer+basil	12.46	1.75	0.14ab	10.62	1.83	2.56	0.60
Basil	11.60	1.82	0.16ab	11.16	0.46	2.26	0.63
Control	13.28	1.61	0.13b	11.4	1.67	2.93	0.60

ns: Not significant, Any means in the same column followed by different letters are significantly ($p < 0.05$) different by *Student-t* test.

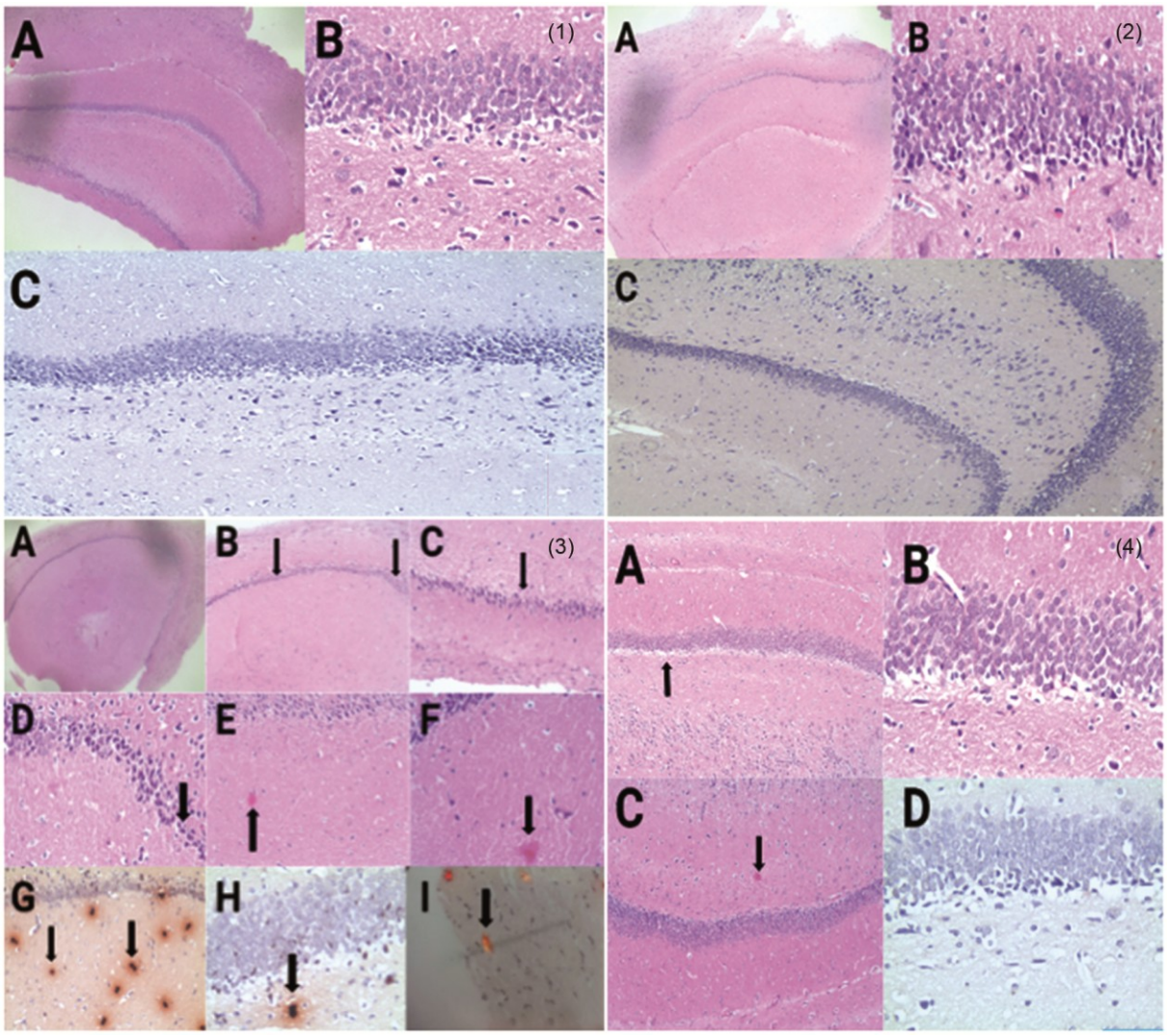

Fig. 2 — Histological image of hippocampus in rats in applications; (1) Control group (A: HEX40, B: HEX400: Regular arrangement of pyramidal neurons, C. Kongo red:X100: Amyloid deposition is not present; (2) Basil group (A: HEX.40, B. HEX400: Pyramidal neurons are regular and not reduced in number, C: Congo red: X100: Amyloid not present; (3) Alzheimer group (A: HEX40, B: HEX100: The decrease in pyramidal cell layer thickness with loss of pyramidal neurons is shown, C: HEX200: Nuclear hyperchromasia, D: Neuronal loss and cytoplasmic vacuolization, E: HEX200: Amyloid deposition, F: HEX400: Amyloid deposition, G: Congo red:X200: Amyloid deposition, H: Congo red: HEX400: Amyloid deposition, I: Congo red: HEX400: Amyloid deposition, apple green birefringent polarization); (4) Alzheimer+Basil group (A: Slight cytoplasmic vacuolization is present in regularly arrayed pyramidal neurons, B: HEX400: No significant reduction in pyramidal neurons C: Presence of focal amyloid, D: Congo red: HEX200, No Amyloid)

hippocampus tissues stained with hematoxylin-eosin (HE). According to the findings obtained in these control groups, pyramidal nerve cells showed a regular distribution in the hippocampus tissues. In addition, amyloid deposition could not be detected. Similarly, as seen in Figure 2.2, regular pyramidal neurons did not decrease in number in the basil groups. However, as seen in Figure 2.3, pyramidal neuron loss has occurred in alzheimer's models. Also, the cell layer thickness at the pyramidal borders was decreased compared to the control groups.

As seen in Figure 2.4 A, pyramidal neurons were arranged in an orderly manner in Alzheimer+basil groups. These neurons have light cytoplasmic fluid-filled spaces. In Figure 2.4 B, it was determined that there was no significant decrease in pyramidal neurons. Focal amyloid was detected in HE stained hippocampus tissues, as shown in Figure 2.4 C. In contrast, amyloid accumulation was not observed in Congo RED stained samples in Figure 2.4 D.

Similarly, in our study, cytoplasmic vacuolization activity was found to be milder in the Alzheimer's +

basil group compared to the Alzheimer's group. In addition, it was observed that the reduction of pyrimidal neurons did not occur significantly in this group. All these findings suggest that basil application may reduce the destructive effect of Alzheimer's pathology on hippocampus tissues.

In this study, unlike the Alzheimer's group, amyloid accumulation was not detected on hippocampal tissues in Congo Red staining in basil group. However, regional amyloid accumulation was detected in histological staining with HE. This result suggests that amyloid immunoreactivity, which varies according to the use of basil in Alzheimer's disease, should be examined with different histological techniques.

Discussion

The Ocimum genus is very complex because of having high morphological variability with genetic diversity. The high diversity in essential oil contents of the basil can show differences depending on the different agroclimatic conditions, cultivation and agronomic techniques across geographic regions[14,23]. The results of our study showed that Dino cultivar had higher essential oil content compared with other genotypes.

In this study, the obtained essential oil content was compared with a study by Karaca *et al.*[24] who noted that nine basil essential oil contents changed between 0.25-1.06%. Likewise, Egata *et al.*[13] reported the the essential oil contents of the Ethiopian sweet basil accessions changed between 0.10-1.02%. In an other study, Yaldiz *et al.*[25] reported that the essential oil ratio of basil grown under different poultry manure application ranged from 0.72% to 1.07%. Also, the esential oil content from 74 different genotypes was found as between 0.04 and 1.71%[13]. The essential oil content of dino cultivar determined in the present study was within the range reported by Yaldiz & Camlica[14] and it was found higher than other researcher findings.

The results of the present study are in a good agreement with those of the study by Elmas *et al.*[26], who reported that methyl chavicol, citral and geranial were the major compounds in *O. basilicum* essential oils.

Similarly, Yaldiz *et al.*[25] reported that the major constituents of basil oil were *p*-Allyl-anisole (5.65-17.90%), nerol (6.69-16.11%), linalool (5.10-10.81%), z-citral (5.23-10.73%), methyl eugenol (5.59-9.27%) and cedrane (0.1-7.90%) which was in partial agreement with this study. Whereas camphor, limonene and b-selinen were found as main essential oil compositions of the basil in Northeast India[27]. Also, Ozcan & Chalchat[28] reported that methyl eugenol, α-cubebene, nerol and epsilon-murolene were the major components of the basil.

The differences in the essential oil components from this study can be explained depending on the different environmental conditions, genetic diversity, chemotypes differences, harvest time and using fertilizer.

Previous studies reported that Aβ leads to high H_2O_2 levels and lipid peroxide accumulation in the cells[29]. In addition, it causes an increase in the oxidant MDA and H_2O_2 levels in brain tissue in a mouse model of AD[30]. Similarly, Júnior *et al.*[31] revealed that basil cultivars belonging to the 'estragole chemotype' may maintain antioxidant capacity, preserving GSH and GPx amounts and decreasing toxic products preserving cellular redox state. It was reported that Aβ caused the decrease in SOD and CAT levels in vivo and in vitro[30,31]. Moreover, significant decreases have been revealed in antioxidants SOD and CAT levels in post mitochondrial supernatant from the postmortem frontal cortex of individuals with AD[32]. Shalan & Alhasan[33] investigated the neuroprotective potential of basil leaf extracts in reducing oxidative stress in rats with AD. The researchers observed a notable decline in serum superoxide dismutase levels accompanied by a significant reduction in serum MDA levels. Additionally, they noted a decrease in tau protein expression. These results are in accordance with those reported by[34], who observed an improvement in oxidative stress status in rats with AD following administration of serum SOD. Furthermore, the researchers observed an elevation in MDA levels in rats subjected to AlCl3-induced injury. Mohammadali *et al.*[35] reported that basil extract delayed the accumulation of Aβ plaques and normalized hippocampal morphology, thus protecting hippocampal tissue from the deleterious effects of hypercholesterolemia and improving learning and memory impairments. Mohd-Zahid *et al.*[36] showed that basil protected neurons against oxidative damage by scavenging free radicals, restoring SOD activity, and preventing cell death. Furthermore, Singh *et al.*[37] assessed the neuroprotective efficacy of basil leaf extract in mice models, identifying a notable reduction in brain infarct size and oxidative stress markers. Similarly,

Oyeniran *et al.*[38] observed significant improvements in locomotor performance in flies supplemented with basil and reported potential therapeutic benefits in alleviating the neurological consequences of AlCl3-induced neurotoxicity. Our findings are consistent with the researchers' findings. These findings provide support for the potential neuroprotective properties of basil.

In this study, we examined amyloid immunoreactivity, pyrimidal neuron number and pyrimidal cell layer thickness in hippocampus tissues related to basil use in Alzheimer's model. The results of the present study compare favourably with previous studies, showing reduced the neurodegenerative and atrophic changes induced in the hippocampus[39]. The results of the current study were closely related to those reported by Gradinariu et al who determined that basil application improved behavioral tests with anxiolytic and antidepressant-like effects. However, the changes in amyloid accumulation were not explained through immunohistochemical analysis[40].

A study from the pevious studies reported that different basil extract doses (100, 200, 400 800 mg/kg of extract) of green *Ocimum basilicum* were used for retention and retrieval of memory in mice. The effective dose was found as 400 mg/kg extract. At the end of the study, it was reported that green *Ocimum basilicum* affects memory-enhancing depending on the contained antioxidant activity of flavonoids, tannins and terpenoids of green *Ocimum basilicum*[15]. The results of our study are in agreement with reported previous studies. Also Farag *et al.*[41] reported that estragole was found to be most active AchE inhibitor followed by cineole, camphor and eugenol, and these compounds bind to key amino acids in the catalytic domain of AchE. In addition, estragole, which is main essential constituent of basil, was potent AchE inhibitors[42], and another compound, limonene, was observed to exert a neuroprotective effect in a Drosophila model of AD induced by Aβ1-42. The compound was observed to reduce ROS production, kinase phosphorylation, neuroinflammation, and cell death, while exhibiting no influence on Aβ1-42 accumulation and aggregation[43]. Therefore, as described above, the active compounds of basil exert their effects by inhibiting AChE, which in turn decreases amyloid aggregation, prevents neuroinflammation and increases brain antioxidant activity. Our results showed similarity with Nemati *et al.*[44], who showed that *Ocimum basilicum* caused a concentration-dependent increase in the percentage of animal entries and the time spent in the open arms, while having no effect on the total distance travelled by the animals or the number of entries into the closed arms.

Conclusions

In this study, our result showed that basil extract affects the memory of the rat positively against AD. Both biochemical and histopathological results revealed that application of the basil extract showed a positive effect and prevented AD. Basil extract and its bioactive components improve AD condition by inhibiting the enzymes involved in neurotransmission, suppressing and oxidative stress. Furthermore, basil can be employed in an additive or synergistic capacity to demonstrate a spectrum of neuroprotective mechanisms, which could prove an efficacious approach in the pursuit of Alzheimer's disease therapeutics.

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Conflict of Interest

The authors have no financial and non-financial conflicts of interest to declare.

Author Contributions

GY has supervised the research work. GY and MC has presented the idea, carried out research work, conceptualization of research, essential oil and its scoposition analysis, wrote manuscript, drafting, reviewing and editing the manuscript and performed data analysis. AC conducted to biochemical analysis and behavioral test. SED carried out the pathology studies.

Ethics Approval

The Animal Research Local Ethics Committee of Bolu Abant İzzet Baysal University approved to this work (approval date: 05.03.2019 and number: 2019/14).

Data Availability

The authors approved that the data supporting the findings of this study are available within the manuscript.

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