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The Moderating Effect of *Hypericum thymbrifolium* against Memory Loss and Alzheimer's Disease (Experimental Study in Mice)

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Abstract

Symptoms of neurodegenerative diseases acquired through neurologic disorder in behavior and memory are sometimes associated with severe aggression. Differences in terminology have resulted in varying estimates, but behavior disorders and memory loss appear to be characteristic of Alzheimer's disease. This work provides a brief neurologic comparison between Alzheimer's model and treated Alzheimer's with *Hypericum thymbrifolium* known as a kind of Turkish tea. The advantages of using phytotherapy against neurodegenerative diseases, including Alzheimer's, reduce the rate of cascade reactions of neurodegeneration and amyloid-beta synthetizes. *Hypericum thymbrifolium* is a species of Turkish antioxidant plant extracted and studied in Istanbul University Faculty of Pharmacy Department of Pharmacognosy. Mice were randomized into 3 groups, 6 mice each. Group 1: control; 2: Alzheimer's model ($AlCl_3$ orally+ IP D-Gal); 3: treated Alzheimer's with the ethanol extract of *H. thymbrifolium.* After 90 days of experimentation, neurologic tests were necessary in order to evaluate neurologic disorders; the results of these tests showed significant differences in behavior and memory between the treated Alzheimer's and Alzheimer's. A histological study was necessary to confirm the neurologic tests and check the nervous tissues state; our results confirmed the decrease of injuries of the pyramidal cells in the cerebral cortex and hippocampus of treated Alzheimer's mice.

Keywords: Alzheimer's disease (AD), antioxidant, *Hypericum thymbrifolium*, mice, phytotherapy

1. Introduction

Alzheimer's disease AD is characterized by a wide range of physical, functional, cognitive, and behavioral disorders s (Moreira P.I. et *al*,2008; Magali Dumont, M. Flint Beal 2011). It usually leads to a marked decrease in the cognitive, mental, and also physical skills of the affected person. In the course of time, the elderly patients with Alzheimer's disease (AD) manifest increasing difficulty in carrying out activities of daily living along with behavioral and psychological symptoms of dementia including signs of disturbed perception, thought content, mood, or behavior like hoarding, wandering, aggression, and disinhibition (Moreira P.I. et *al*. 2008; Magali Dumont M.and Flint Beal 2011).

Alzheimer's disease (AD) is one of the consequences of bioavaibility of prooxidant, exhibited various symptoms corresponding to cerebral impairments such as loss of concentration and short term memory (Teresa M. et *al*., 2006).

Unfortunately, some xenobiotics are used daily in some nutrients, cosmetics, additives (dyes, anti-coagulants, firming…), in cooking utensils and in pharmacological agents including antacids and antiperspirants, from which prooxidants enter to the human body at supraphysiological doses, thus increasing the concentration of prooxidant in the blood dramatically (ex: aluminum)(Markesberry and Carney, 1999).

Naturally, the brain is abundant in antioxidants that control and prevent the detrimental formation of reactive oxygen species (ROS) generated via Fenton chemistry involving redox-active metal-ion reduction and activation of molecular oxygen (Esra Eroglu et *al*., 2018).

Some species of *Hypericum* are known in turkey as a kind of tea that can be used to improve some cases of depression. It would be of great interest to find out whether food supplements endowed with antioxidative potential could prevent/reverse or reduce neurological alterations. Experimentally, the treated Alzheimer's and Alzheimer's model is often used for pathological and pharmacological investigations (Rodger L. et *al*., 2017).

In this study, the effect of *H.thymbrifoliumon* on Alzheimer's model was investigated. The behavior and memory responses are used in order to evaluate its positive effects. The histological study is a complementary part that gives a cellular and tissular explanation of these improvements.

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2. Material & methods

2.1. Plant material

The specimens of flowering aerial parts of *H. thymbrifolium* were collected from their natural habitats on the roadsides nearby the town of Malatya located in the East Anatolia Region of Turkey. The plant materials were identified by Prof. Dr. Şükran Kültür and voucher specimens were deposited in the Herbarium of the Istanbul University Faculty of Pharmacy, Istanbul, Turkey (ISTE number is 93194).

2.1.1. Preparation of the extract

The dried flowering aerial parts of the plant (10 g) were macerated in ethanol (100 mL) for 3 days at room temperature at dark, and filtered through Whatman No-1. The residue from the filtration was extracted again twice using the same procedure. The filtrates were combined and then evaporated to dryness under reduced pressure at a temperature below 45 °C. The crude ethanol extract was lyophilized and stored at -20 °C. (Esra Eroglu et *al.* 2018).

2.2. Animals

Studies were performed using young adult mice (3-month-old, 22-26 g) housed in the Laboratory Animal Care of Mostaganem under a 12-hour light-dark cycle, with *ad libitum* access to food and water. Mice were obtained from PASTEUR Institute of Algiers

Mice were assigned into three groups, each containing six animals. Control Group: mice were administered freshwater orally and served as normal control. Alzheimer's model Group: mice were treated with $AlCl_3$ (100 mg/kg/day) concomitant with the IP of D-Galactose (D-Gal) in order of 200 mg respectively of the volume of (0,1mL/day). Treated Alzheimer's group: received concomitant with the Aluminum and D-Galactose dose, (0,1 mL) IP of *H. thymbrifolium* dried ethanol extract in order of 200 mg/kg/day. The experiment lasted for 3 months.

2.3. Chemical& treatment

$AlCl_3$ and D-Gal were purchased from Sigma-Aldrich Chemicals.

Both the dried ethanol extract of *H.thymbrifolium*, and the chemicals ($ALCl_3$& D-Gal) were dissolved in distilled water, in order to be administrated for the needed dose.

2.4. Neurologic tests

2.4.1. Behavioral tests

Disturbance of behavior is enduring and creates severe difficulties for people with neurodegenerative diseases. Neurobehavioral disability (NBD) is a term that has been evolved to highlight the combination of neurological and neuropsychological origins of behavior disorders observed amongst people with this neurologic injury (Per M. Roos et *al.*, 2006).

2.4.1.1. Stress: Forced swimming test

Forced swimming tests included two sessions, once each 24 h. On the first day, each mouse was placed individually in a glass cylinder (22 cm in diameter, 40 cm high) filled with water, kept at 25°C, at a depth of 20 cm. Animals were forced to swim, and immobility time was recorded. The mouse was considered as immobile when it stopped struggling and moved only to remain afloat, keeping its head above the water (Persolt, R.D. et *al.*, 1977).

2.4.1.2. Curiosity

Curiosity is one of the behavioral tests to evaluate the exploration properties of animals using a hole (deep hole test); on the other hand the anxiety would be known with the high score of this hole platform test, the visit of the hole is the score for this test.

2.4.1.3. Morris water maze

After 3 months, spatial memory was measured by the Morris water-maze (MWM) test. The water maze consisted of a circular water tank (160 cm in diameter and 35 cm in height), which was divided by four fixed points on its perimeter to four quadrants. It contained an escape platform of 10 cm in diameter of the same color as the rest of the basin (to eliminate any false-positive results due to vision), placed in a constant point of the basin throughout the trials and kept 1.5 cm below the water surface. Mice were placed at a start point in the middle of the rim of a quadrant not containing the escape area with their face to the wall. Animals had four trials per day separated by 10 minutes for 5 successive days, during which the times required to find the hidden platform were averaged.

2.4.1.4. Memory Test: Radial Arm Maze

The radial arm maze was designed for evaluating spatial learning and memory in rodents. The maze has eight arms radiating from a central platform. A small food site is at the end of one arm. The design ensures that, after checking the food site, the animal is always forced to return to the central platform before making the next choice. We used this maze to evaluate the degree of memory loss in the AD mouse model with or without drug treatment.

The maze test consisted of a 4-days training session and a 1-day testing session. Before the training session, mice were kept on a restricted diet in order to motivate the mice to seek food in the maze.

During the testing session, mice explored the maze baited at arms, and a 5-min period was provided to each mouse. Each reentry (entry to a previously visited arm) and error (entry to a non-baited arm) was recorded to evaluate working memory (short term) and reference memory (long term), respectively.

To demonstrate that mice were matched for memory ability after the treatment period, working memory and reference memory were calculated by averaging the reentries and error entries, respectively, for the 5 days of testing. To compensate for any baseline differences between Alzheimer's treated groups (the ethanol extract of *Hypericum thymbrifolium*), a regression method was used to compare the memory of Alzheimer's & Alzheimer's treated groups.

2.5. Statistical Analyses:

The data are expressed as means with S.E.M. The statistical significance of differences between groups was assessed with an analysis of variance followed by Student Newman-Keuls. A P value of 0.05 or less taken as a criterion for a statistically significant difference.

2.6. Histological study

In order to confirm the previous tests, the histological study was necessary. Mice were sacrificed, brains were quickly removed. Segments were fixed in Formol solution

and paraffin-embedded. Serial sections of 2µm were obtained with a Leica microtome. For histologic observation, deparaffinized sections were stained according to conventional histological and histochemical stains (H&E).

3. Results & Discussion

Obtained results have suggested that *Hypericum thymbrifolium* had an improvement on the behavioral, and memory tests clearly showed significant differences between the treated Alzheimer's and Alzheimer's model.

3.1. Behavioral tests

Conditioned avoidance response is an experimental model to study procedural type of behavior in curiosity, forced swimming test; which tested the mice performance; these tests show a remarkable difference between the treated Alzheimer's and the Alzheimer's group; the Persolt test was assessed by the immobilized time represented as a despairing time.

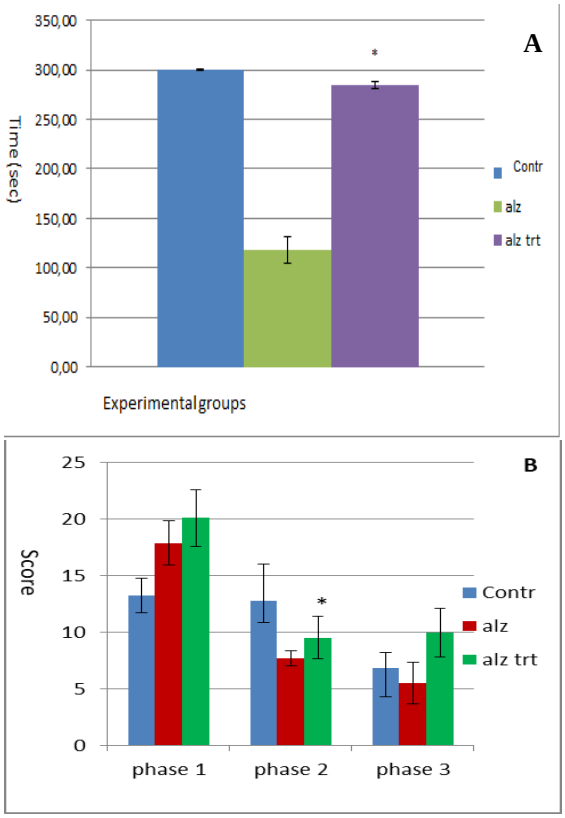

Figure1: A: forced swimming test: Control mice (contr), Alzheimer's model (alz) by $AlCl_3$ (100 mg/kg) Or& D-Gal (200mg/Kg)IP and the Treated Alzheimer's with the ethanol extract of *H. thymbrifolium* (200mg/kg) orally for three months. **B**: curiosity test: Control mice (contr), Alzheimer's model (alz) by $AlCl_3$ (100 mg/kg)Or& D-Gal (200mg/Kg) IP and the Treated Alzheimer's with the extract (200mg/kg) orally for 3 months.

3.2. Memory tests

The obtained results of the Spatial Memory test preferably conditional, during the experimental tests showed that Alzheimer's mice take much longer to reach the food in the arm lit unlike control mice, treated Alzheimer's, and that put a very short time to get informed on the arm (Figure 2).

The test of long term memory, represented with Morris maze, showed a significant decrease in the retention of the learned task was observed in Alzheimer's mice, Whereas the treated mice with the ethanol extract of *H. thymbrifolium* where it had shown a significant results $P<0.05$ noted as score per time, 5mn each day showed high activity for the Alzheimer's treated comparatively with the intoxicated group (Figure 2).

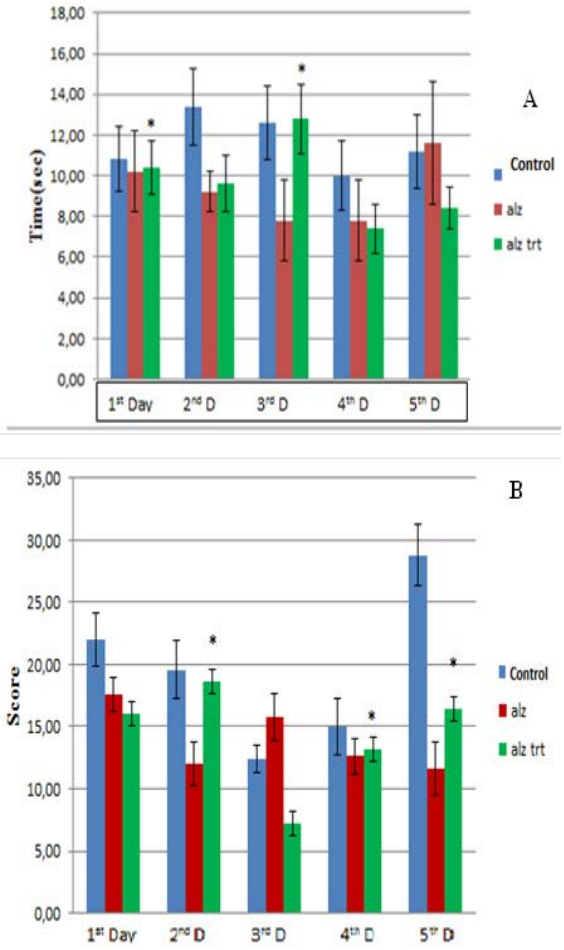

Figure 2: **A:** Work Spatial Memory; Morris aquatic test: Control mice(contr), Alzheimer's model (alz) by $AlCl_3$ (100 mg/kg)Or& D-Gal (200mg/Kg) IP and the Treated Alzheimer's with the ethanol extract of *H. thymbrifolium* (200mg/kg) orally for three months. **B**: Reference Spatial Memory; maze 8 arms test: Control mice(contr), Alzheimer's model (alz) by $AlCl_3$ (100 mg/kg)Or& D-Gal (200mg/Kg) IP and the Treated Alzheimer's with the ethanol extract of *H. thymbrifolium* (200mg/kg) orally for 3 months

3.3. Histological studies

Histological statute of nervous tissues of treated Alzheimer's in H&E staining shows that there are typical neuropathological changes in the cerebral cortex and hippocampus of Alzheimer's model, whereas the treated Alzheimer's tissues showed a shrunken decreased in all brain compartments, shows moderated neuropathological changes improved by *Hypericum thymbrifolium* administrated in parallel of $AlCl_3$.

In the control groups, the neurons were full and arranged tightly, and the nuclei were light stained. By comparison with the Alzheimer's model, the cytoplasm of neurons were shrunken, the nuclei were side moved and

dark stained, neurofibrillary degeneration and neuron loss were well identified in Alzheimer's group observed in the cerebral cortex in addition to the observed effect in the hippocampal tissues.

Hypericum thymbrifolium administration induced the neuron's protection showed in characteristic shape conservation, with reducing neurofibrillary tangles (Figure 3).

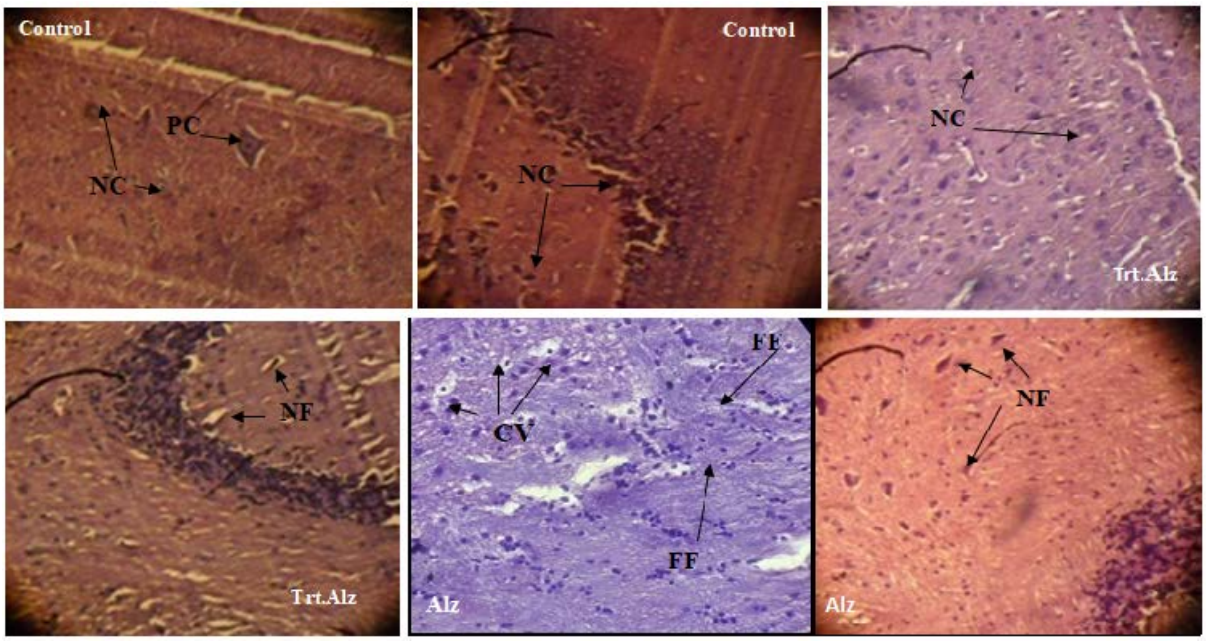

Figure 3: Microscopic study of nervous tissues of 2µm performed by H&E staining in cerebral cortex& Hippocampus of control mice(Contr);Alzheimer's mice $AlCl_3$ orally (100 mg/kg) and treated Alzheimer's mice(alz.trt) with the ethanol extract of *H. thymbrifolium*(200 mg/kg) for 3 months(G×400).(alz) cerebral cortex & Hippocampus characterized by a decrease in cell density and neuronal vacuolization(G×400), treated Alzheimer's(alz.trt) shows a decrease of vacuolization and a normal cells density in (G×400).**NC**: Normal Cell; **PC**: Purkinje cell; **CV**: Cell Vacuolisation; **NF**: Neurofibrillary tangles; **FF**: Fibrillary Form

4. Discussion

Neurodegenerative diseases are largely spread without any difference in age; they affect the young people as well as the old and are characterized by progressive pathological changes in the brain that translate into clinical signs of decline in cognitive abilities (memory), functional abilities, mood, and behavior. This pathogenesis is the result of the invasion of some contaminants, including heavy metal which we can classify Aluminum.

Alzheimer's pathological changes in the brain are characterized by deterioration and loss of neurons (nerve cells) leading to brain atrophy (Rodger L. et *al*., 2017).

Some solutions are actually used, but they could not resolve that neuronal loss, else the phytotherapy showed a large improvement and decrease the rate of Amyloid beta reaction cascade.

Antioxidant effects of some dietaries cooperate with the body enzymes to protect the brain from free radical damage (Piccaglia, R. et *al.,* 1998). *Hypericum thymbrifolium* is largely used in turkey as a kind of tea, Esra el *al* found that this species is very rich in phenolic compounds (Trifunovic S. et *al*., 1998;Radulovic N. et *al*.,2007; Esra Eroglu et *al* 2018), which report a rate of this latter of 20.7 mg / g dry weight (DW). In the same previous study, the *in vitro* antioxidant activity was measured by the lipoperoxidase inhibitory power LPO test as well as the scavenging of the free radical DPPH; the results gave a relevant capacity on the LPO (4.39 ± 0.08^{a}) as well as DPPH scavenging (0.622 ± 0.051^{a}) at 50% concentration (Esra Eroglu et *al* 2018). That potent antioxidant activity is involved in the *in vivo* results. This result is explicated physiologically by increasing antioxidant enzymes and the important anticholinesterase activity (63.41 ± 3.29a) (Esra Eroglu et *al.,* 2018), that conduced to a mental improvement at behavior and memory capabilities (Olton DS . et *al.,* 1981; Sahin, G. et *al.,* 1994), and as another benefit, this extract also have low or no side effects (Esra Eroglu et *al.,* 2018 ; Olton DS . et *al.,* 1981; Sahin, G. et *al.,* 1994).

Alzheimer's was induced by Aluminum chloride ($AlCl_3$) orally at 100 mg/kg/day in drinking water with IP D-Gal (200mg/kg), another group Alzheimer's model was treated with the ethanolic extract of *H.thymbrifolium* orally (200 mg/kg/day) and the control group received drinking water only during 3 months.

In this investigation, the effect of *H.thymbrifolium* against Alzheimer's disease explained with behavior improvement as the memory and learning in mice tested by neurologic experiments (Olton DS. Et *al*.,1981) In the forced swimming test, the recorded immobility time is reduced in the Alzheimer mice compared to the other groups of mice, knowing that the immobility time in treated Alzheimer group is very close to the control group which coincides with the result of Sahin et *al*. (Sahin G. et *al*., 1994). The hole test was used to evaluate the exploration behavior exhibited by the Alzheimer's mice; for this purpose it was noted during the test that the Alzheimer's model mice are less exploratory than the treated Alzheimer's and control mice, contrary to what was found in Djebli & Rebai's work (Djebli N. and Rebai W.2008). The working spatial memory test showed that the control groups and the treated Alzheimer are more motivated than the Alzheimer's; the reference spatial memory (RSM)of control and treated Alzheimer's mice are

improved, the difference was shown by spending a short time finding the platform in the first four days unlike the Alzheimer's group (Bizon J.L. et *al*., 2009). In the same side of the *in vivo* study, we noted in histological study, a reducing in the fibrin form of amyloïd beta in brain tissue, conduced to enhancing behavior, memory improvement and reduction of neurotoxicity in Alzheimer's disease that appeared in contrary with decreasing of cell density, deformity in tissue and fibrillary form of cell deposits (amyloid-beta). These brain moderation changes by *H. thymbrifolium* were explained by reducing oxidative damage which contributes to disease pathogenesis and AChE inhibition, were in accordance with the aim of this study which is to prove the antioxidant effect of the selected species against Alzheimer's disease, appeared in behavioral, memory and histological studies (Esra Eroglu et *al* 2018; Müller WE. 2006; Langosch, J. M. et *al*., 2002).

5. Perspectives

The mechanism of the response and the level of this moderating effect are not investigated in this study; that still needs other *in vivo* and *in vitro* studies in order to understand this treatment effect, than valorizing it to resolve health problems, which could not be resolved with the chemical treatment.

Conflict of interests

There is no conflict of interests.

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