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Original Article

Effects of Wormwood Essential Oil on Rat with Alzheimer's Disease

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ABSTRACT

Background and Aim: The purpose of the present study is to provide a basis for the use of Wormwood essential Oil as a therapeutic agent for Alzheimer's Disease (AD). **Methods:** The rat's model of AD induced by Ibotenic (IBO) acid and the effect of wormwood oil was evaluated through the measurement of cognitive functions using the Morris Water Maze (MWM) and oxidative stress and histomorphometric analyze in hippocampus tissue. **Results:** In rat treated with wormwood oil (50 mg/kg) cognitive functions were significantly enhanced and were nearly returned to normal in the treated group. The levels of MDA in hippocampus tissue were significantly elevated, while antioxidant markers (CAT, GSH, and SOD) were reduced in model group compared to controls. The administration of wormwood oil improved all these parameters. In particular, oil led to increase pyramidal neuron counts at CA1 and CA3 compared with model. **Conclusion:** Our data provide that wormwood essential oil may be useful for management and treatment of AD.

Keywords: Wormwood (*Artemisia absinthium* L), Essential oil, Alzheimer's disease, Experimental, Histomorphometric analysis.

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INTRODUCTION

Alzheimer's Disease (AD) is a multifarious neurodegenerative disease that causes cognitive impairment and gradual memory loss. The extracellular aggregation of an Amyloid-β peptide (Aβ) as senile plaque and the intraneuronal deposition of neurofibrillary tangles owing to abnormal hyperphosphorylation of tau protein, massive cholinergic neuronal death, an inflammatory cascade, and oxidative stress all represent specific neuropathological features of AD.[1] The hippocampus is most vulnerable to these pathological alterations and affected than other areas of the brain.[2] Until now, all the drugs available for AD only relieve clinical symptoms but are unable to prevent progression or to replace the degenerated neurons.[3] Consequently, the development of novel therapies to alleviate AD pathologies, inhibit neuronal death, replace dead neurons, eliminate toxic deposits, and provides a better niche for remaining cells are very necessary. Traditional medicine has become more highly regarded in the past few last decades. *Artemisia absinthium* L. (Asteraceae), commonly known as wormwood in United Kingdom and absinthe in France, is an aromatic, perennial small shrub. The herb has always been of a great botanical and pharmaceutical interest and is employed in folk medicine against various paints.[4] Wormwood essential oil has been widely used mainly for its neuroprotective,[5] antifungal,[6] antimicrobial,[7] insecticidal,[5] acaricidal,[8] anthelmintic,[9] antimalarial,[10] hepatoprotective[11] and antidepressant[12] proprieties. It was always used as a drink in France called absinthe which caused dementia. In the 18th century, alcoholic decoctions of wormwood and other plants were used as all-purpose remedies or "cure-alls," but it was not until the beginning of the 19th century that the wormwood-flavoured alcoholic extracts and distillates were seen not only as potent medicines, but also as aperitifs, and the large-scale production of absinthe began. In addition, *Artemisia absinthium* L. was used freshly for the plant species, as well as for the alcoholic beverage. Korea has long cultivation history of several wormwood species. Few studies have reported biological components of wormwood essential oil for the treatment of many diseases but there has been no research to use its oil as herbal medicine for the treatment of AD.

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MATERIALS AND METHODS

Animals

Adult Wistar rats (11-12-week-old, 250~300g) were provided by Laboratory Animal Centre of the Pyongyang University of Medical Sciences. 45 rats were randomly chosen and divided into three groups (Normal: non-manipulation, Model: IBO acid injection and Experiment: wormwood oil administration). During the experiment, feed and water were available to rats at any time. The temperature was maintained at 20±2°C and the humidity was 55%. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Basic Medicine, Pyongyang University of Medical Sciences. After 1 week of acclimatization to the cage, 15 rats of experimental group were orally administrated with wormwood essential oil at daily doses 50mg/kg for 10 weeks from one week later after injection with IBO acid.

Preparation of Wormwood Essential Oil Extract

Wormwood (*Artemisia absinthium* L.) essential oil used in this study was kindly extracted by Traditional Medicine Centre of Pyongyang University of Medical Sciences. The oil extraction was performed using hydro distillation process following the described protocol by Tigrine *et al*[13] and the oil was kept at room temperature till be used. As described by manufacturer, the wormwood oil contained about 32.8% 1,8-cineole, 5.8% camphor, 25.1% borneol and other unidentified compounds.

AD Model

AD rat model was performed by bilateral IBO acid (Sigma, St. Louis, MO) injection into the rat's hippocampus. IBO acid was dissolved in 10mM artificial cerebrospinal fluid at a concentration of 8 mg/mL.[14] The surgery protocol was the same as that explained in previous study.[15] To anesthetize the rats, a mixture of ketamine (80 mg/kg body weight) and Xylazine (10 mg/kg body weight) was intraperitoneally administered. Each rat was carefully placed in a stereotactic frame under complete aseptic conditions.

Intra-hippocampus injection of 4 µL of IBO acid solution was made slowly over 5 min using a 10 µL Hamilton syringe (coordinate, ML: 2.5mm, AP: 3.5mm, and VD: 2.7mm relative to the bregma) according to the brain atlas of Paxinos and Watson.[15] Following the injection, the skin was sutured, and the rats were returned to their cages when fully recovered from anesthesia.

Morris Water Maze Test (MWMT)

After acclimatization to the environment for one week, the rats were subjected to the Morris water maze to assess spatial memory and learning. The methodology of this test was completely explained by Vorhees *et al.*[17] The animal's behavior, the escape latency time (time needed to reach to platform), and time in the target quadrant (quadrant time) were tracked and measured with a digital camera. The scores were recorded on the 6th day after training (before IBO injection) and at the end of the study (before sample collecting with one week).

Sample Collection

Samples were concurrently collected from each group 11 weeks after IBO acid injection (10 weeks after wormwood oil administration). Rats were euthanized, and their brains were dissected. The hippocampus was collected from each rat. Tissue samples were divided into two groups. The 1st group was fixed in neutral formalin 10% (48 hr) for histological examination and morphometric analyses and the 2nd group was homogenized and cold centrifuged; the supernatants were separated and carefully collected into clean sterile tubes to be used in the evaluation of antioxidants and oxidative stress parameters.

Antioxidants and Oxidative Stress Parameters

Catalase (CAT) activity was assessed in hippocampus tissues according to the method of Claiborne,[18] while Glutathione (GSH) level was determined according to the method of Jollow DJ *et al.*[19] and Superoxide Dismutase activity (SOD) was measured according to Marklund *et al.*[20] Furthermore, Malondialdehyde (MDA) level was determined according to the technique of Todorova I *et al.*[21] All other parameters were determined in hippocampus tissue homogenates using a spectrophotometer.

Histomorphometric Analysis

Formalin-fixed hippocampus samples were processed for paraffin wax embedding by dehydration in ascending grades of ethanol, then cleared in two changes of xylene; they were paraffin wax impregnated and embedded, sectioned with a rotatory microtome and mounted on coated glass slides. The mounted sections were stained with Hematoxylin and Eosin (H&E) stain and examined under a light microscope. For morphometric analysis, morphometric measures were obtained from all groups. Five different samples from five different rats were taken. Three H&E-stained sections from each sample were examined under higher magnification (x400). The mean number of their viable neurons at CA1 and CA3 was also counted.

Statistical Analysis of Data

Results were expressed as the mean and SEM. Data were analyzed by one-way Analysis of Variance (ANOVA) using SPSS 16.0 and the differences between the means assessed using Dunnet's multiple range test. A P value of < 0.01 was taken as the level of statistical significance.

Table 1: The effects of extract on cognitive functions.

	Latency time (s)	Quadrant time (s)
Normal	19.5±1.4	26.7±2.3
Model	44.3±2.9**	8.2±0.6**
Experiment	28.1 ± 1.7ΔΔ	17.3± 1.1ΔΔ

Each value represents the mean ± SEM of 15 rats per group. **P<0.01 as compared with normal group. ΔΔP<0.01 as compared with model group.

Table 2: The effects of oil on oxidative stress in hippocampus.

	GSH (mg/g tissue)	MDA (nmol/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)
Normal	12.4±0.6	65.2±3.9	578.2±15.8	2.2±0.3
Model	3.1±0.2**	142.5±7.2**	321.9±10.4**	0.6±0.1**
Experiment	8.7± 0.5ΔΔ	108.8± 4.6ΔΔ	502.8± 12.5ΔΔ	1.7± 0.2ΔΔ

Each value represents the mean ± SEM of 15 rats per group. **P<0.01 as compared with normal group. ΔΔ P<0.01 as compared with model group.

RESULTS

Cognitive Functions

As shown in Table 1, in experimental group, the indices related to cognitive functions such as the latency and quadrant time was significantly improved compared with model group (P<0.01).

Level of GSH and MDA, Activities of SOD and CAT

Table 2 showed that in experimental group, oxidative stress-associated indices including GSH, MDA, SOD and CAT was significantly improved compared with model group (P < 0.01).

Pyramidal Neuron Count in Hippocampus

As shown in Figures 1 and 2, administration of oil to rats significantly increased the viable neuronal numbers at CA1 and CA3 area in hippocampus compared to model rats.

DISCUSSION

Currently, the European Prevention of Alzheimer's Dementia (EPAD) committee has classified AD as the most widespread neurodegenerative disease, and its prevalence is expected to double over the next 20 years.[22] The Ibotenic acid (IBO) induced AD model has been chosen for this study as intrahippocampal injection of IBO in rats produces nearly the same symptoms and pathological changes that are seen in humans with AD.

Wormwood (*Artemisia absinthium* L.) selected in present study has been used as an herbal medicine for the treatment of some diseases, in particular, liver diseases such as chronic hepatitis, cirrhosis and AFL (Alcoholic Fatty Liver) and is also one of the

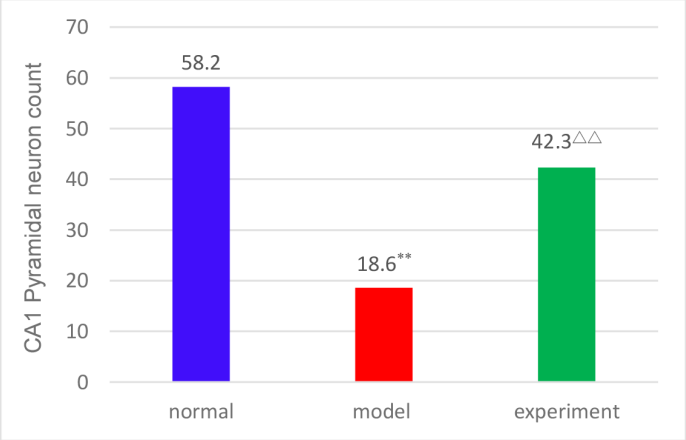

Figure 1: CA1 viable neuronal number.

Each value represents the mean ± SEM of 15 rats per group. **P<0.01 as compared with normal group. ΔΔP<0.01 as compared with model group.

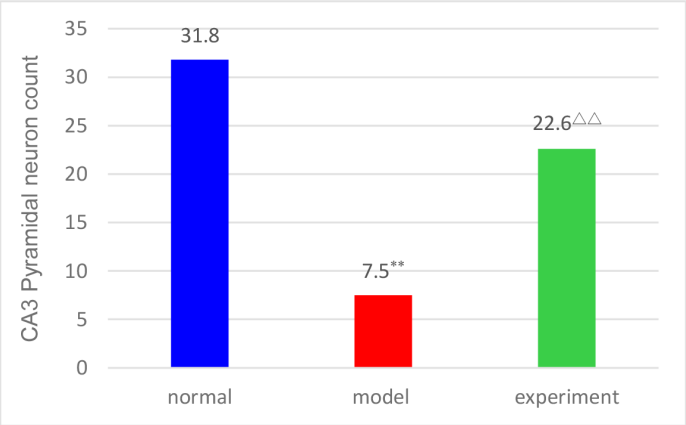

Figure 2: CA3 viable neuronal number.

Each value represents the mean ± SEM of 15 rats per group. **P<0.01 as compared with normal group. ΔΔP<0.01 as compared with model group.

aromatic plants that are widely used as a potent source of natural antioxidants in DPR of Korea. Aromatic and medicinal plants are an easily accessible and edible source of natural antioxidants.

The present study was thus performed to determine whether wormwood essential oil can have protective effects on damaged neurons resulting from the IBO injection. In our study, we investigated the protective effects of wormwood oil on cognitive responses and oxidative stress in the case of AD. Our results showed that administration of wormwood oil significantly decreases the latency and, while increasing quadrant time. According to previous studies, aromatic and medicinal plants have the ability to protect the organism from damage caused by free radical-induced oxidative stress such as cancer and cardiovascular and neurodegenerative diseases.[23,24] As mentioned in current studies for AD, the accumulation of Aβ resulted in activation of astrocytes. The activated astrocytes enhance the production of ROS. Therefore, Aβ is linked with the

formation of ROS and induction of oxidative stress that occurs in the case of AD.[25] The significant reduction in antioxidant levels (GSH, SOD, CAT) corroborates the idea that oxidative stress is the early event that has a crucial role in the disease progression. Additionally, the reduction in GSH, SOD, and CAT levels may be linked to loss of neurons in AD and increasing the free radical load, which triggers oxidative stress.[26] In our study, the hippocampal activities of antioxidant molecules were significantly restored with wormwood oil administration, compared to the IBO model group. We suggest that this was attributed to the main active component of wormwood oil (1,8-cineole, camphor, borneol, etc.,) exhibiting greater free radical scavenging activity. The histological findings and morphometric analysis of the hippocampus confirmed the neurotoxic effects of IBO as the numbers of viable pyramidal neurons at CA1 and CA3. Our results are in harmony with the results of Karthick *et al.*[14] who reported that a significant histological alteration associated with a significant increase in dead neurons was detected at CA1 and CA3 after IBO injection. In the current study, the neuroprotective ability at CAI and CA3 of hippocampus was detected in rats administrated wormwood oil. Loss of neurons at CA1, CA3 after intra hippocampus injection with IBO was associated with a progressive decline of cognitive function and production of oxidative stress. So that, we suggest that the effect of wormwood oil on the histomorphometric analysis may be also linked to cognitive functions and relief of oxidative stress in hippocampus and this essential oil has the positive therapeutic effects on AD induced by IBO in rats.

CONCLUSION

Our results suggest that wormwood oil used in the present study have potential for the treatment of AD. However, further studies can confirm these effects and also investigate the potential components of wormwood oil that play a definite role in pathophysiology of AD. It is however assumed that wormwood oil might provide a beneficial effect in the treatment of AD.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AD: Alzheimer's Disease; **IBO:** Ibotenic Acid; **MWM:** Morris Water Maze; **Aβ:** Amyloid-β Peptide; **CAT:** Catalse; **GSH:** Glutathione; **SOD:** Superoxide Dismutase; **MDA:** Malondialdehyde; **EPAD:** European Prevention of Alzheimer's Dementia.

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