International Journal Islamic Education, Research and Multiculturalism
(IJIERM)
Available online https://journal.yaspim.org/index.php/IJIERM/index

Effect of Andaliman (*Zanthoxylum Acanthopodium DC*) Extracts on The Histological Display of Lewy Body in The Brain of Rotenone Induced Wistar Rats

Lubna Afina
Email: lubna.afina@gmail.com
Prima Indonesia University, Medan, Indonesia

Binarwan Halim
Email: binarwan2002@yahoo.com
Prima Indonesia University, Medan, Indonesia

Linda Chiuman
Email: lindachiuman@unprimdn.ac.id
Prima Indonesia University, Medan, Indonesia

Wenti Anggraeni
Email: wentianggraeni@unprimdn.ac.id
Prima Indonesia University, Medan, Indonesia

Kartika Sari Rizki
Email: kartikasarizki@unprimdn.ac.id
Prima Indonesia University, Medan, Indonesia

Abstract: Dementia Lewy Body (DLB) is a form of dementia characterized by abnormal accumulation of α-synuclein protein in the brain, forming lewy bodies that damage the function of neurons and nervous system. This study aimed to evaluate the effect of andaliman fruit extract (Zanthoxylum Acanthopodium DC), which is rich in antioxidants, on the number of lewy bodies in the brain of rotenone-induced Wistar male rats. Histology analysis was performed to observe the number of lewy bodies in the brain. The results showed a significant decrease in the number of lewy bodies in the treatment group compared to the positive control group ($p<0.05$). Increasing the dose of andaliman extract was directly proportional to the decrease in the number of lewy

bodies, with the highest dose (450 mg/kgBB) providing the best protective effect. These findings suggest that the antioxidant content in andaliman extract can neutralize the oxidative stress caused by rotenone, thus protecting neurons from damage. This study supports the development of andaliman extract as an alternative therapy to prevent or inhibit the progressivity of DLB.

Keywords: *Neuroprotective, Lewy Body, Dementia, Rotenone, Andaliman*

INTRODUCTION

Dementia with Lewy bodies (DLB) is a neurodegenerative disease characterized by impaired cognitive function due to abnormal accumulation of α-synuclein protein.[1] The condition resembles alzheimer's and parkinson's diseases, causing challenges in early diagnosis. Oxidative stress is a major contributing factor to DLB, occurring when levels of reactive oxygen species (ROS) exceed the body's antioxidant capacity, resulting in damage to brain cells and accelerating the neurodegeneration process.[2]

In addition to environmental factors, genetic factors play an important role in the development of DLB. SNCA mutations increase α-synuclein protein aggregation, while GBA dysfunction disrupts lysosomal autophagy, leading to abnormal α-synuclein accumulation. The interaction of these genetics with oxidative stress, for example due to exposure to environmental toxins such as rotenone, accelerates the progression of the disease. Rotenone, a substance found in South America and Southeast Asia, is now an active ingredient in various pesticides.[3] Its high lipophilic nature allows penetration to the blood-brain barrier. Rotenone inhibits complex I of the mitochondrial electron transport

[1] Valentina Tjandra Dewi and Anak Agung Ayu Putri Laksmidewi, "Manifestasi Klinis dan Gambaran Pencitraan Struktural dan Fungsional Berbagai Subtipe Demensia," *Cermin Dunia Kedokteran* 50, no. 11 (November 1, 2023): 594–599.

[2] Tiago Fleming Outeiro et al., "Dementia with Lewy Bodies: An Update and Outlook," *Molecular Neurodegeneration* 14, no. 1 (December 2019): 5.

[3] Ramdiana Ramdiana and Legiran Legiran, "Literature Review: Stres Oksidatif dan Reproduksi Wanita," *Jurnal Ilmu Kesehatan Masyarakat* 12, no. 03 (May 2, 2023): 202–214.

chain, causing a decrease in ATP and an increase in ROS that contribute to oxidative stress.[4] Epidemiological studies suggest that exposure to pesticides such as rotenone may increase the risk of neurodegenerative diseases, including lewy body formation.[5]

Research conducted by Betarbet et al. 2000 successfully demonstrated that rotenone administration in mice can trigger Parkinson's disease.[6] Mice are often used as research models to study cognitive symptoms because they have similar biological responses to humans, especially in terms of toxin metabolism, which is more relevant compared to mice.[7] In 2020, the United States Environmental Protection Agency (EPA) banned the use of rotenone in food and only allowed its use as a piscicide under strict supervision. Since the implementation of this regulation, cases of lethal rotenone poisoning in humans have become very rare and only a few have been recorded.

The mechanism of rotenone in triggering DLB development is through oxidative stress and mitochondrial dysfunction. By inhibiting mitochondrial complex I, rotenone increases ROS and changes the conformation of α-synuclein protein. The increase in ROS leads to oxidation and nitration modifications in α-synuclein, which accelerates fibril and lewy body formation. Mitochondrial dysfunction also weakens protein degradation systems such as ubiquitin-proteasome and autophagy, resulting in misfolded α-synuclein stacking. Oxidative stress in neurons occurs when ROS production exceeds the capacity of the antioxidant system, producing superoxide (O_2-) and hydrogen peroxide (H_2O_2). Superoxide triggers the formation of nitric oxide (NO), which reacts into

[4] Hasanuddin University et al., "Perbandingan Kadar Reactive Oxygen Species (ROS) sebagai Penanda Stres Oksidatif pada Ibu Hamil Dengan dan Tanpa Vaginosis Bakterial," *Indonesian Journal of Obstetrics & Gynecology Science* 6, no. 2 (July 17, 2023): 281–287.

[5] Dedianto Hidajat, Febry Gilang Tilana, and I Gusti Bagus Surya Ari Kusuma, "Dampak Polusi Udara terhadap Kesehatan Kulit," *Unram Medical Journal* 12, no. 4 (December 31, 2023), accessed December 18, 2024, https://jku.unram.ac.id/index.php/jk/article/view/1021.

[6] Ranjita Betarbet et al., "Chronic Systemic Pesticide Exposure Reproduces Features of Parkinson's Disease," *Nature Neuroscience* 3, no. 12 (December 2000): 1301–1306.

[7] Institutes National, "Lewy Body Dementia: Information for Patients, Families, and Professionals" (2023).

peroxynitrite (ONOO-), a compound that damages neurons. Peroxynitrite also forms nitrotyrosine, which promotes aggregation of insoluble α-synuclein, damages neurons, and triggers neurodegenerative diseases such as DLB. Therapeutic strategies with antioxidants are becoming important to reduce oxidative stress in the progression of DLB.[8]

Motor impairment in DLB is related to the accumulation of misfolded α-synuclein in the substantia nigra in the central nervous system. The buildup of lewy bodies in this area causes damage to dopaminergic neurons, resulting in significantly reduced dopamine levels.[9] This damage continues as long as oxidants and free radicals remain formed in the cells. Therefore, additional antioxidants are needed to reduce oxidative stress. Antioxidants play a role in restoring oxidized glutathione (GSSG) to the active form of glutathione (GSH), which is responsible for regulating redox balance in cells and preventing further damage.[10]

Natural antioxidants are found in various parts of plants, such as vegetables, fruits, leaves, stems, and roots.[11] Active compounds contained in fruits, such as phenolic acids, flavonoids, carotenoids, stilbenoids, and fiber, play an important role in protecting the body from the damaging effects of free

[8] maria Faustina Jesslyn Herlianto, Siufui Hendrawan, And Frans Ferdinal, "Uji Fitokimia Dan Kapasitas Total Antioksidan Ekstrak Daun Salam (Syzygium Polyanthum)," *Jurnal Kesehatan Tambusai* 4, no. 4 (December 16, 2023): 5012–5018.

[9] Prasetyo Tri Kuncoro, Indarwati Setyaningsih, and Moh Was'an, "Peran α-synuclein sebagai target terapi parkinsonisme pasca cedera kepala" 19, no. 1 (2020).

[10] Nyoman Ayu Anindya Maharani et al., "The Potential of Natural Levodopa in Koro Benguk Seeds (Mucuna pruriens) for Treatment in Parkinson's Disease," *Jurnal Biologi Tropis* 24, no. 3 (July 27, 2024): 341–363.

[11] Wilna Iznilillah, Aji Jumiono, and Muhammad Zainal Fanani, "Perbandingan Proksimat, Antioksidan, dan Antosianin pada Berbagai Produk Olahan Pangan dengan Penambahan Pewarna Alami Bunga Telang," *Jurnal Ilmiah Pangan Halal* 5, no. 2 (October 22, 2023): 163–174.

radicals while providing significant health benefits. This diversity of compounds makes plants a highly valuable natural source to support a healthy lifestyle.[12]

One fruit that has health benefits and has been widely researched is andaliman fruit (Zanthoxylum acanthopodium DC). The andaliman plant is known to have various medicinal properties thanks to its rich content of bioactive compounds, especially antioxidants such as flavonoids.[13] The andaliman fruit, the most commonly utilized part, has a round shape with a diameter of about 2-3 mm. The fruit is green when unripe, turns reddish when ripe, and blackens when dried. Each fruit contains one seed.[14] The flavonoids contained in andaliman have strong antioxidant properties, which also provide anti-inflammatory effects, help reduce inflammation, and support overall health. Apart from flavonoids, andaliman also contains other bioactive compounds such as alkaloids, saponins, and terpenoids. These compounds contribute to various health benefits, such as boosting the immune system, improving blood circulation, and fighting infections. The combination of active compounds in andaliman provides great potential to support human health, both in traditional and modern phytochemical-based medicine.[15]

Although early research shows the potential of andaliman as a promising source of natural antioxidants, there are some important limitations to consider.

[12] Khairun Nisa et al., "Pengaruh Pemberian Ekstrak Andaliman Terhadap Jumlah Hematokrit, MCV, MCH dan MCHC Pada Jenis Tikus Putih (Rattus nervogecius L.) Yang Di Induksi Boraks," *JURNAL RISET RUMPUN ILMU HEWANI* 1, no. 2 (December 19, 2022): 52–65.

[13] Micella Tanessa et al., "EFFECTIVENESS OF ANDALIMAN EXTRACT NANOEMULSION (ZANTHOXYLUM ACANTHOPODIUM DC) AGAINST LIPID PROFILE IN STREPTOZOTOCIN-INDUCED WISTAR MALE RATS (STZ)," *Journal Health & Science : Gorontalo Journal Health and Science Community* 7, no. 1 (January 14, 2023): 27–34.

[14] Ira Syaputri, Ermi Girsang, and Linda Chiuman, "Test of Antioxidant And Antibacterial Activity of Ethanol Extract of Andaliman Fruit (Zanthoxylum Acanthopodium Dc.) With Dpph (1.1-Diphenyl-2-Picrylhydrazil) Trapping Method And Minimum Inhibitory Concentration," *International Journal of Health and Pharmaceutical (IJHP)* 2, no. 2 (February 14, 2022): 215–224.

[15] Micella Tanessa et al., "EFFECTIVENESS OF ANDALIMAN EXTRACT NANOEMULSION (ZANTHOXYLUM ACANTHOPODIUM DC) AGAINST LIPID PROFILE IN STREPTOZOTOCIN-INDUCED WISTAR MALE RATS (STZ)," *Journal Health & Science : Gorontalo Journal Health and Science Community* 7, no. 1 (January 14, 2023): 27–34.

To date, clinical evidence regarding the effectiveness of andaliman in the prevention and treatment of neurodegenerative diseases, particularly Dementia with Lewy bodies, is still very limited. Most existing studies have focused on the identification and characterization of bioactive compounds, while the understanding of the specific mechanisms of how these compounds interact with the pathological pathways of DLB still needs to be deepened.[13]

Andaliman has potential as a DLB therapy through three main mechanisms. First, andaliman flavonoids capture free radicals and increase the activity of antioxidant enzymes, reducing oxidative stress which is the main cause of neuronal damage in DLB. Second, its bioactive compounds can inhibit α-synuclein aggregation, both through direct interaction with this protein and by modulating the cellular environment. Third, andaliman's anti-inflammatory effect protects dopaminergic neurons in the substantia nigra from oxidative and inflammatory damage. This combination of mechanisms makes andaliman a potential candidate for natural ingredient-based therapy for DLB. Validation of the therapeutic potential of andaliman requires comprehensive studies. In vivo studies with rat models exposed to rotenone can be used to test the neuroprotective effects of andaliman extract and its active compounds. Molecular mechanisms, including interactions with the glutathione system and the impact on α-synuclein aggregation, also need to be further analyzed. Controlled clinical studies are needed to evaluate the safety, effectiveness, bioavailability, optimal dose, as well as potential side effects of andaliman in humans. In addition, the development of formulations that enhance the absorption and activity of andaliman's bioactive compounds should be a priority. This research will provide a strong scientific basis for future andaliman-based DLB therapy.[15]

THEORETICAL BASIS

In neurodegenerative diseases, mitochondrial dysfunction and free radical-induced oxidative stress are the main factors contributing to cellular damage. One of the neuropathological characteristics is the formation of abnormal α-synuclein protein in the cytoplasm of neuronal cells, which can be found in

several areas of the brain.[16] In addition, chronic inflammatory processes also play an important role in the pathogenesis of the disease, which exacerbates neuronal damage and accelerates neuronal degeneration.[17] Dementia with Lewy bodies (DLB) is often described as a synucleopathy, caused by abnormal accumulation of α-synuclein and intracellular aggregates that form lewy bodies. The α-synuclein protein itself is a presynaptic nerve terminal protein encoded by the SNCA gene. This protein functions to modulate synaptic vesicle recycling as well as neurotransmitter release, which is vital for the normal function of the nervous system.[18]

The main symptoms of Dementia with Lewy Body (DLB) include dementia with significant cognitive fluctuations, vivid visual hallucinations, and signs of parkinsonism in the early stages of the disease.[19] In DLB, impaired executive function and visuospatial abilities are often more prominent, while verbal memory impairment is relatively more preserved when compared to Alzheimer's. Although both are types of dementia that affect memory, the main difference lies in the more typical clinical manifestations in DLB, such as more dramatic fluctuations in cognition and visual hallucinations that often occur in the early stages, while in Alzheimer's, cognitive impairment is more consistent and progressive.[20]

Neuropathological features in neurodegenerative diseases, especially DLB and Parkinson's disease, show abnormal deposition of α-synuclein in the cytoplasm of neuronal cells in several brain areas. These α-synuclein aggregates

[16] Mamlikatu Ilmi Azizah, "Penyakit Mitokondria: Review" (2020).

[17] Andrea Bianca Castafiore Kusuma et al., "Uji Kapasitas Antiokisdan pada Ekstrak Daun Peppermint (Mentha piperita L.) dengan Metode DPPH, FRAP, ABTS," *Jurnal Sehat Indonesia (JUSINDO)* 6, no. 02 (July 23, 2024): 878–886.

[18] Airi Tarutani et al., "Potent Prion-like Behaviors of Pathogenic α-Synuclein and Evaluation of Inactivation Methods," *Acta Neuropathologica Communications* 6, no. 1 (December 2018): 29.

[19] Rana Nadiyah Adwinda and Fariani Syahrul, "Faktor yang Berhubungan dengan Kejadian Demensia : Literature Review: Factors that are Associated with the Occurrence of Dementia : Literature Review," *Media Publikasi Promosi Kesehatan Indonesia (MPPKI)* 6, no. 1 (January 13, 2023): 12–19.

[20] Outeiro et al., "Dementia with Lewy Bodies."

are known as lewy bodies.[21] Filamentous α-synuclein is a protein that is widely expressed in the brain and can form amyloid-like filamentous structured aggregates. In the early stages of the disease, lewy bodies are found in the cholinergic and monoaminergic neurons of the brainstem and olfactory system. As the disease progresses, these deposits may spread to limbic regions and the neocortex.[22] The main risk factor for DLB is age, with prevalence increasing with age. In addition, lifestyle is also considered a risk factor, although evidence linking specific lifestyles to an increased risk of DLB is limited. Some studies suggest that a healthy lifestyle, such as regular exercise, mental stimulation, and a healthy diet, may help reduce the risk of diseases related to aging, including dementia.[23]

The pathology associated with lewy body accumulation is known to be related to degradation of dopaminergic neurons in the substantia nigra, which is often associated with oxidative stress due to increased oxidants. This may exacerbate the disease progression of Dementia with Lewy Body (DLB). A growing body of evidence suggests a link between α-synuclein aggregation and oxidative stress. In studies conducted on the SH-SY5Y cell line, treatment with low doses of rotenone inhibited electron transport chain complex I, leading to increased production of reactive oxygen species (ROS) and increased α-synuclein levels.[24] Therefore, rotenone is classified as a class II pesticide by the World Health Organization (WHO), which means the chemical is moderately hazardous. Although rotenone poisoning in humans is very rare, effective

[21] Arianna Novati et al., "Rat Models of Major Neurodegenerative Disorders," *Ageing and Neurodegenerative Diseases* 2, no. 4 (2022): 17.

[22] Kuncoro, Setyaningsih, and Was'an, "Peran α-synuclein sebagai target terapi parkinsonisme pasca cedera kepala."

[23] Syifa Fitriyanda Salsabila, Widhya Aligita, and Yani Mulyani, "Review: Neuroprotective effect of herbal plant extracts against Parkinson's disease," *Jurnal Ilmiah Farmasi* 17, no. 2 (December 28, 2021): 198–209.

[24] Tarutani et al., "Potent Prion-like Behaviors of Pathogenic α-Synuclein and Evaluation of Inactivation Methods."

intravenous doses are difficult to receive, as the absorption of rotenone through the skin or lungs is very low.[25]

According to research by Mayazaki et al., 2020 reported that exposure to low doses of rotenone (2 mg/kg/day) through subcutaneous infusion with a mini pump for 1 month can increase hyperphosphorylation of Tau, α-synuclein, amyloid-beta peptide, and protein carbonylation in the hippocampus, substantia nigra (SN), and locus coeruleus in old Lewis rats.[26] In general, clinical signs of rotenone poisoning in animals include pharyngitis, gastric pain, vomiting, muscle tremors, chronic convulsions, and respiratory stimulation followed by depression. [27] Andaliman contains flavonoid compounds that have strong antioxidant activity, which is beneficial to the health of the body. These flavonoids play an important role in protecting body cells from damage caused by free radicals, which can accelerate the aging process and contribute to the development of various degenerative diseases.[28] In Anggraini et al.'s 2020 study, a daily dose of 300 mg/kg BW for eight weeks was able to reduce memory impairment, reduce the rate of aging, and prevent cognitive decline in rats given D-galactose to induce aging.[29] The potential of andaliman to treat DLB can be explained through three main mechanisms. First, the antioxidant activity of andaliman flavonoids plays a role in neutralizing ROS triggered by mitochondrial complex I inhibition, as seen in the rotenone model. This mechanism is important considering that studies on SH-SY5Y cells show that increased ROS directly affect α-synuclein levels.^18^ Second, andaliman's

[25] Chuanqi Chu et al., "Lactobacillus Plantarum CCFM405 against Rotenone-Induced Parkinson's Disease Mice via Regulating Gut Microbiota and Branched-Chain Amino Acids Biosynthesis," *Nutrients* 15, no. 7 (April 1, 2023): 1737.

[26] Ikuko Miyazaki and Masato Asanuma, "The Rotenone Models Reproducing Central and Peripheral Features of Parkinson's Disease," *NeuroSci* 1, no. 1 (August 5, 2020): 1–14.

[27] Danielle F. Mello et al., "Rotenone Modulates Caenorhabditis Elegans Immunometabolism and Pathogen Susceptibility," *Frontiers in Immunology* 13 (February 22, 2022): 840272.

[28] Tanessa et al., "Effectiveness Of Andaliman Extract Nanoemulsion (Zanthoxylum Acanthopodium Dc) Against Lipid Profile In Streptozotocin-Induced Wistar Male Rats (STZ)."

[29] Novati et al., "Rat Models of Major Neurodegenerative Disorders."

antioxidant effects may protect dopaminergic neurons in the substantia nigra, an area that undergoes significant degeneration in DLB due to lewy body accumulation.^19^ Third, andaliman's ability to prevent cognitive decline, as shown in the D-galactose-induced rat model.^20 Although preliminary results show promising potential, several limitations need to be considered in the development of andaliman as a DLB therapy. Firstly, existing studies have mostly focused on the general effects of antioxidants, while the specific mechanism of interaction of andaliman compounds with DLB pathology, especially its effect on α-synuclein aggregation, still needs further investigation. Second, although there is evidence of andaliman's effectiveness in aging models, in vivo studies using specific DLB models, such as rotenone-induced mice, are needed.[30] to validate its neuroprotective effects. Third, translational research and clinical trials are needed to determine the bioavailability of andaliman's active compounds in the brain, the optimal dose for therapeutic effects, and its safety profile in DLB patients. These investigations will provide a strong scientific foundation for the development of andaliman-based therapies in the management of DLB.

RESEARCH METHODS

This research is an experimental design research with the Complete Randomized Design (RAL) method,[31] which was carried out at the Faculty of Veterinary Medicine, Syiah Kuala University in the period July - September 2024. A total of 25 male Wistar rats, weighing 160-200 grams and aged 8-12 weeks. This study has obtained ethical approval from the Prima Indonesia University Health Research Ethics Committee with letter number 036/KEPK/UNPRI/2024. The andaliman fruit extract was obtained through an extraction process by maceration

[30] Novati et al., "Rat Models of Major Neurodegenerative Disorders."

[31] Hanifa Ira Agustin, "Analisis Kadar Protein, Kadar Zat Besi Dan Sifat Organoleptik Pada Mi Kering Dengan Substitusi Kacang Tolo (Vigna unguiculata)," *Pontianak Nutrition Journal (PNJ)* 7, no. 1 (March 31, 2024): 461.

method using 70% ethanol, which resulted in a concentrated extract.[32,33] A total of 25 male Wistar rats were acclimatized for 7 days under standard laboratory conditions with room temperature of 22±2°C, relative humidity of 55±10%, 12-hour light/dark cycle, and ad libitum access to standard feed and drinking water. Mice were placed in ventilated plastic cages with 4-5 animals per cage and then divided into five groups: negative control, positive control, and three treatment groups. The negative control group was only given distilled water, while the positive control group and three treatment groups were given rotenone 3 mg/kgBB subcutaneously every other day for 14 days to induce lewy body formation. After 14 days of rotenone induction, on day 15, each treatment group was given andaliman fruit extract at different doses: a dose of 150 mg/kgBB in treatment group 1, a dose of 300 mg/kgBB in treatment group 2, and a dose of 450 mg/kgBB in treatment group 3.

The administration of the extract was carried out every day for 14 consecutive days. On the 28th day, after the administration of the extract, the rats were euthanized and the brain was taken for histopathological examination. The rat brains were then stained using hematoxylin-eosin (HE) staining, which examination focused on the substantia nigra, cerebral cortex, and hippocampus areas. Brain samples were cut coronally with a thickness of 5 µm using a microtome. Lewy body identification was performed at 400x magnification with criteria: round or oval-shaped eosinophilic cytoplasmic inclusions with a clear halo. For each area examined, observations were made in at least 5 different fields of view. A score of 0 indicates that no pathology was found, indicating healthy brain tissue. A score of 1 indicates the presence of a few Lewy bodies or Lewy neurites, indicating the beginning of lewy body formation. A score of 2 reflects the presence of two or more Lewy bodies per microscope field and few Lewy

[32] Winda Irawati Zebua, Linda Chiuman, and Edy Fachrial, "Histopathological Evaluation of Green Betel Leaf Extract Ointment on Incision Wounds Infected with Staphylococcus Aureus in Wistar Rats" (2024).

[33] Supri Hartini, I Gede Andika Sukarya, and Anatasya Putri Wasito, "Comparative Analysis of Sharia and The Effects of Black Garlic (Black Allium Sativum) on Creatinine Levels in Tuberculosis-Infected Mice (Mus Musculus)" 6, no. 3 (2024).

neurites. A score of 3 indicates more significant enhancement, with more than four Lewy bodies and scattered Lewy neurites. A score of 4 represents the most severe condition, where there are many Lewy bodies and Lewy neurites widely scattered throughout the observed brain tissue. This classification helps in assessing the severity and progressivity of the disease associated with lewy body formation.[34]

Data analysis uses the SPSS program to process the data obtained. The data normality test will be carried out using the Shapiro-Wilk test with a criterion of $p > 0.05$, which aims to ensure that the data is normally distributed.[35] To test whether there is a significant difference between the experimental groups, one-way analysis of variance (One Way ANOVA) is used with a confidence level of 95% ($p < 0.05$). This technique uses a comparison of means between treatment groups to determine whether there is a statistically significant difference. If the ANOVA test results show a p value <0.05, it can be concluded that there is a significant difference between the experimental groups.[36]

The research flow chart can be seen in the following figure;

[34] Per Borghammer et al., "Neuropathological Evidence of Body-First vs. Brain-First Lewy Body Disease," *Neurobiology of Disease* 161 (December 2021): 105557.

[35] Wiwik Wiwik, Muh. Tawil, and Arie Arma Arsyad, "Efektivitas Penerapan Media Audio Visual untuk Meningkatkan Hasil Belajar IPA Peserta Didik Kelas VII SMPN 21 Sinjai," *JURNAL PENDIDIKAN MIPA* 12, no. 4 (December 14, 2022): 1131–1138.

[36] Zebua, Chiuman, and Fachrial, "Histopathological Evaluation of Green Betel Leaf Extract Ointment on Incision Wounds Infected with Staphylococcus Aureus in Wistar Rats."

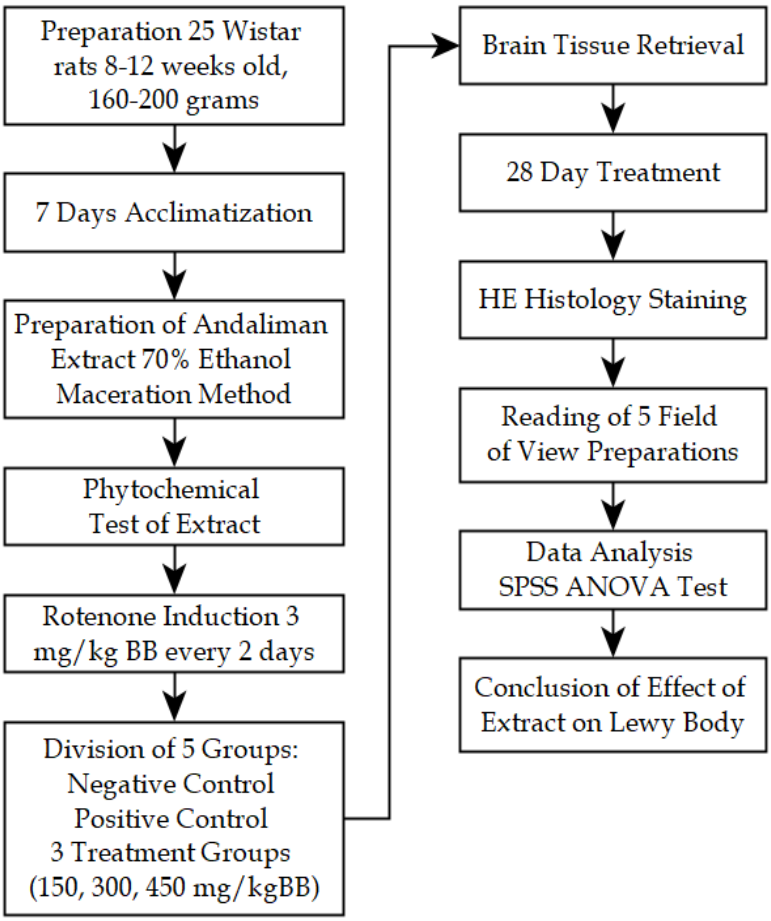


Figure 1: Research flow chart

RESULTS AND DISCUSSION

Results

1. Phytochemical Test Results of andaliman fruit Extract

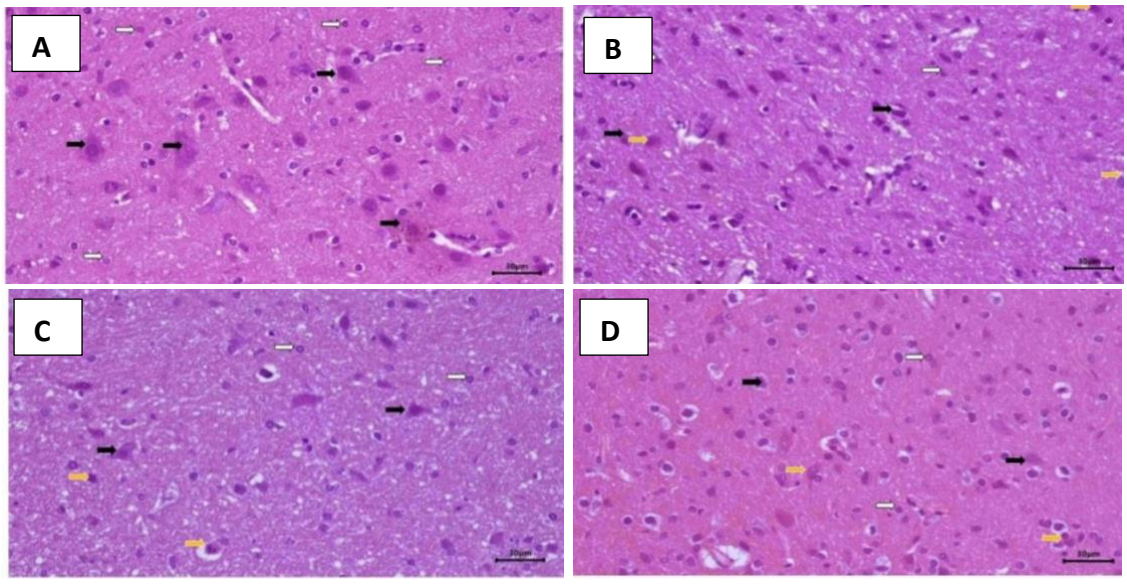
Table 1. Phytochemical screening of andaliman fruit extracts

Secondary Metabolite Compounds	Reagents	Result
Alkaloids	Bouchardart	+
	Maeyer	+

	Dragendroff	+
	Wagner	+
Terpenoids/steroids	Salkowsky	+
	Lieberman-Burchad	+
Saponins	Aquadest + 96% Alcohol	+
Flavonoids	Mg(s)+HCl(p)	+
	FeCL3 5%	+
	10% NaoH	+
	H2SO4	+

Phytochemical screening of andaliman fruit extract showed the presence of alkaloid, terpenoid/steriod, saponin, and flavonoid compounds, which were detected using various reagents. All of these compounds gave positive results, indicating that andaliman fruit extract contains secondary metabolites that have the potential to provide strong antioxidant effects, while other contents support biological activities such as anti-inflammatory and neuroprotective.

2. Histopathology Analysis of Rat Brain



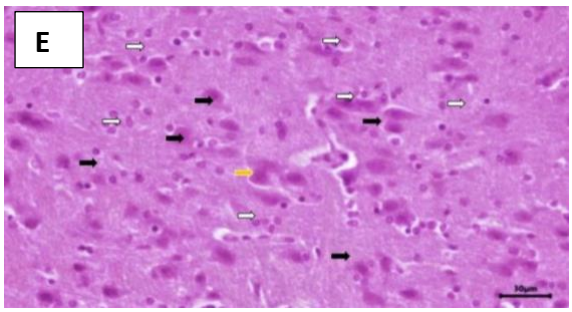


Figure 2. Histopathology of rat brain

Histopathological analysis of the negative control group (Figure A) showed well-preserved neuronal morphology, with large neurons and basophilic cell nuclei, indicating high metabolic activity. Astroglia also appeared normal, with no indication of significant neuronal degeneration, necrosis, or inflammation. In comparison, in the positive control group (Figure B), there was obvious neuronal degeneration with eosinophilic intracytoplasmic inclusions, or lewy bodies, which are characteristic of dementia disease with lewy bodies. This indicates the toxic effect of rotenone on brain tissue.

In treatment 1 (Figure C), neuronal degeneration was significant, although there were neurons with normal morphology. Eosinophilic inclusions known as lewy bodies were also found, accompanied by neuronal necrosis and nuclear pyknosis, indicating a progressive neurodegenerative process. In treatment 2 (Figure D), neuronal degeneration was significant with similar findings of lewy bodies and neuronal necrosis. Although some neurons and astroglia still appeared normal, neuronal damage was quite extensive.

In treatment 3 (Figure E), there was a clear picture of neurological degeneration, with normal-looking neurons but lewy body inclusions suggestive of impaired α-synuclein proteinopathy. Neuronal necrosis with nuclear pyknosis also reinforced the suspicion of an active neurodegenerative process, corresponding to lewy body formation and neuronal death. Overall, all treatment groups showed profound pathological changes, with progressive indications of neurodegeneration.

3. Normality Test (Saphiro-Wilk) Number of Lewy Bodies

Table 2. Normality Test (Saphiro-Wilk)

Tests of Normality			
	Shapiro-Wilk		
	Statistic	df	Sig.
Negative Control	0.684	5	0.470
Positive Control	0.902	5	0.421
Behavior 1	0.813	5	0.103
Behavior 2	0.684	5	0.357
Behavior 3	0.883	5	0.325

Based on the results of the normality test using the Shapiro-Wilk test, it was found that the data in all study groups were normally distributed. For the negative control group, the p value was 0.470, which was greater than 0.05, in the positive control group, with a p value of 0.421, in treatment group 1, the p value was 0.103, treatment group 2 showed a p value of 0.357, and treatment group 3 with a p value of 0.325.

4. Homogeneity test (levene test) Number of Lewy bodies

Table 3. Homogeneity test (levene test)

Test of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
Control + Treatment	Based on Mean	32.332	1	23	0.362
	Based on Median	13.249	1	23	0.233
	Based on Median and with adjusted df	13.249	1	16.084	0.212
	Based on trimmed mean	30.983	1	23	0.209

The results of the variance homogeneity test with Levene's Test showed that the variance between treatment groups was homogeneous, because all significance values (p) were greater than 0.05, namely 0.362, 0.233, 0.212, and

0.209. With this test result, the homogeneity of variance was met for the next analysis.

5. One Way ANOVA Test Number of Lewy Bodies

Table 4. One Way ANOVA Test

ANOVA					
Control + Treatment					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.383	1	2.383	2.924	0.000
Within Groups	18.744	23	0.815		
Total	21.126	24			

The One Way ANOVA test results show that there is a significant difference between the control group and the treatment group. The F value obtained is 2.924 with a significance value (p) of 0.000, which is smaller than 0.05. This indicates that there is a significant difference between the tested groups, so the null hypothesis that there is no difference between groups can be rejected.

DISCUSSION

Phytochemical screening of andaliman fruit extracts showed the presence of various useful secondary metabolite compounds, such as alkaloids, terpenoids/steroids, saponins, and flavonoids. Alkaloid compounds were detected through testing with Bouchardart, Maeyer, Dragendorff, and Wagner reagents which all gave positive results. Terpenoids and steroids were also identified using the Salkowsky and Lieberman-Burchard tests. Saponins were tested with distilled water and 96% alcohol, while flavonoids were detected through reactions with magnesium, HCl, $FeCl_3$, NaOH, and H_2SO_4, all of which showed positive results. The content of these compounds supports the benefits of andaliman fruit extract as a potential antioxidant and neuroprotective agent,

contributing to the protection of body cells from oxidative damage and supporting nervous system health.

This study provides evidence that andaliman fruit extract has significant benefits as an antioxidant and neuroprotective agent, as evidenced through the improvements seen in rat brain cells. The andaliman fruit extract contains various phytochemical compounds with excellent potential to protect nerve cells from damage. In addition, the results showed that the administration of andaliman fruit extract in doses of 150 mg/kgBB, 300 mg/kgBB, and 450 mg/kgBB provided different improvements in rat brain cells. Histological examination at a dose of 450 mg/kgBB provides the most significant and highest improvement in the condition of rat brain cells, when compared to lower doses, thus indicating that higher doses provide a stronger neuroprotective effect.

Andaliman is known to have various biological activities that are beneficial to health, including as a larvicide, anti-inflammatory, analgesic (pain reliever), antimicrobial, antioxidant, and antifungal. The active compounds in andaliman, such as flavonoids and alkaloids, are believed to provide diverse health benefits. Flavonoids, contained in andaliman fruit extract, have strong antioxidant activity, which plays an important role in protecting body cells from free radical damage.[37] This damage can accelerate the aging process and contribute to the development of various degenerative diseases. With this ability, the flavonoid compounds in andaliman are extremely beneficial for improving the overall health of the body.[38]

According to Fikry et al.'s study, 2022 showed that brain damage caused by rotenone was not only in the nigrostriatal pathway, but also caused significant degenerative changes in several other parts of the brain, such as the cerebral

[37] Azzuhra Permata Khaira et al., "Hepatoprotective Effects Of Several Doses Of Nanoherbal Andaliman Extract (Zanthoxylum Acanthopodium) On Liver Function And Histopathology In Streptozotocin- Induced Diabetic Male Wistar Rats For 2 Weeks" 6, no. 3 (2024).

[38] Dian Pratiwi et al., "BIOAKTIVITAS ANTIOKSIDAN ANDALIMAN (Zanthoxylum acanthopodium) SEBAGAI TANAMAN ENDEMIK SUMATERA UTARA" 31, no. 2 (2024).

cortex and hippocampus, which had been subjected to histological examination.[39] These findings are in line with Abdel-Salam et al.'s 2014 study that identified degenerative changes in various brain sites, including the cerebrum, striatum, hippocampus, and substantia nigra, due to increased oxidative stress induced by rotenone.[40] Rotenone, which is derived from the roots, seeds, and stems of tropical plants such as Derris, Tephrosia, Lonchocarpus, and Mundulea, has been shown to affect synucleinopathy models in vitro, by increasing phosphorylation of α-synuclein upon exposure to rotenone and ferrous iron. In neurodegenerative diseases, mitochondrial dysfunction and oxidative stress elicited by free radicals are major factors contributing to cellular damage. One of the most striking neuropathological signs is the formation of abnormal α-synuclein protein in the cytoplasm of neuronal cells in several areas of the brain. Sustained inflammatory processes also play a crucial role in the pathogenesis of the disease, exacerbating neuronal damage and accelerating neuronal degeneration.[29]

Dementia with lewy bodies (DLB) and Parkinson's disease have important similarities in terms of histopathology, where lewy bodies are a major finding. Lewy bodies are intracellular cytoplasmic aggregates composed of proteins and other materials, which are commonly found in dopaminergic neurons in the substantia nigra. The formation of lewy bodies is thought to be triggered by a gene mutation involving the protein α-synuclein, which leads to the aggregation and formation of water-insoluble fibrils in neuronal cells. This buildup of α-synuclein contributes to various symptoms of DLB, such as cognitive fluctuations, visual hallucinations, motor impairments similar to Parkinson's, and sleep disturbances. In addition, changes in brain fluid distribution and impaired brain function can exacerbate the degenerative process, accelerate disease progression, and cause impairments in thinking, movement, behavior, and

[39] Heba Fikry, Lobna A. Saleh, and Sara Abdel Gawad, "Neuroprotective Effects of Curcumin on the Cerebellum in a Rotenone-induced Parkinson's Disease Model," *CNS Neuroscience & Therapeutics* 28, no. 5 (May 2022): 732–748.

[40] Omar Abdel-Salam et al., "Rotenone-Induced Nigrostriatal Toxicity Is Reduced by Methylene Blue," *Journal of Neurorestoratology* (May 2014): 65.

attitude. The early symptoms of DLB are often similar to those of Alzheimer's dementia, which makes it difficult to diagnose correctly.[27]

In the examination of the number of Lewy bodies, the negative control group showed a stable average value of the variable throughout the 28 days of observation. In contrast, the positive control group experienced a significant increase over the period. The treatment groups showed more varied and significant results. In Treatment Group 1, the mean value of the variable remained stable with little fluctuation, indicating that the effect of the treatment was relatively constant. In Treatment Group 2, there was a significant increase in the tested variables, which supports the hypothesis that this treatment can reduce lewy bodies. Treatment Group 3 showed consistent improvement, indicating a sustained positive effect throughout the observation. Overall, this study reinforces the potential of andaliman fruit extract as a neuroprotective agent. Several recent studies have revealed that when neuronal cells suffer damage or protein accumulation, they aggregate in one area to form specialized structures, reducing the impact of the damage.[29] This study also showed that the administration of andaliman fruit extract at a dose of 450 mg/kgBB gave better results than the doses of 150 mg/kgBB and 300 mg/kgBB in increasing brain activity, indicating an increase in the expression of brain activity after high dose administration.

CONCLUSION

This study proves that andaliman fruit extract (Zanthoxylum acanthopodium DC) has a significant effect in reducing neurodegenerative damage in rotenone-induced rats. Through histology test and statistical analysis (One Way ANOVA) with a significance value of 0.000, the study showed that the administration of andaliman fruit extract at doses of 150, 300 mg/kgBB significantly reduced the number of Lewy Bodies in the rat brain, and 450 mg/kgBB as a better dose in increasing brain activity. The results indicate the potential of andaliman fruit as a neuroprotective therapy that can help reduce the risk of neuronal damage, which potentially has important implications in the prevention and treatment of neurodegenerative diseases such as dementia with Lewy Bodies.

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