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Effect of (*Zanthoxylum Acanthopodium DC*) Extract on the Increase of Brain Cells in The Substantia Nigra of Wistar Rats That are Rotenone-Induced

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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder due to the decline of dopaminergic neurons in the substantia nigra, with motor and non-motor symptoms. Oxidative stress, inflammation, and mitochondrial damage play a role in its pathogenesis. andaliman fruit (*Zanthoxylum acanthopodium* DC), rich in alkaloids and flavonoids, has potential as an antioxidant with neuroprotective effects. The purpose of the study was to determine the effect of andaliman fruit extract (*Zanthoxylum acanthopodium* DC) on the increase of brain cells in the substantia nigra of rotenone-induced Wistar rats as a Parkinson's model. This research uses an experimental quantitative study with a *post-test control group design.* Data were analyzed using One Way ANOVA with a significance level of $p < 0.05$. The results showed that 70% ethanol extract of andaliman fruit (*Zanthoxylum acanthopodium* DC) can reduce the number of necrosis cells in the brain. On the 29th day, the average number of necrosis cells in each group was 0.4±0.54 (K-), 4.8±0.83 (K+), 4.4±1.14 (P1), 3.4±0.54 (P2), and 1.2±0.44 (P3). The administration of extracts at doses of 150 mg/kgBB, 300 mg/kgBB, and 450 mg/kgBB can increase the regeneration of brain cells that experience necrosis. Histopathological observations showed that 70% ethanol

extract of andaliman fruit improved the histological structure of brain cells in the substantia nigra area.

Keywords: Parkinson's, *Zanthoxylum acanthopodium* DC, Cell necrosis, Substantia nigra

INTRODUCTION

Parkinson's disease is a neurodegenerative disorder that affects the central nervous system, especially the part of the brain that controls body movement. The disease is characterized by symptoms such as tremors, muscle stiffness, difficulty walking, and impaired balance.[1] Parkinson's disease is caused by damage to dopaminergic neurons (dopamine-producing nerve cells) in the substantia nigra pars compacta area, resulting in impaired body movement and coordination. The disease occurs due to damage to nerve cells in the basal ganglia and loss of neurons in the substantia nigra (brain stem) that produce dopamine.[2] Dopamine plays an important role in regulating the coordination of body movements. When dopamine levels decrease, stimulation to dopamine receptors in the striatum decreases, causing symptoms such as tremors, slowed movement, muscle stiffness and impaired balance.[3]

The decline of dopaminergic neurons in Parkinson's is closely linked to oxidative stress in the brain due to the buildup of free radicals. Excessive free radicals can damage cell membranes, proteins and DNA. This condition can be exacerbated by exposure to substances such as rotenone (a pesticide) which

[1] Deni Kurnia et al., "Seleksi Fitur dengan Particle Swarm Optimization pada Klasifikasi Penyakit Parkinson Menggunakan XGBoost," *Jurnal Teknologi Informasi dan Ilmu Komputer* 10, no. 5 (October 17, 2023): 1083–1094.

[2] Prasetiawan Eka, Sabri Emita, and Ilyas Syafruddin, "Gambaran Histologis Hepar Mencit (Mus Musculus L.) Strain Ddw Setelah Pemberian Ekstrak N-Heksan Buah Andaliman (Zanthoxylum Acanthopodium Dc.) Selama Masa Pra Implantasi Dan Pasca Implantasi" (2012).

[3] Ilsa Hunaifi et al., "Edukasi Deteksi Dini Penyakit Parkinson Pada Kader Puskesmas Dalam Rangka Hari Parkinson Sedunia," *Jurnal Abdi Insani* 9, No. 3 (September 19, 2022): 1012–1018.

disrupts mitochondrial function and increases free radical production.[4] Current Parkinson's treatment focuses on three main strategies: increasing dopamine levels, stimulating dopaminergic activity, and reducing dopamine degradation. While these treatments are effective at relieving symptoms and improving quality of life, none have been able to stop the progression of the disease. For advanced cases that are unresponsive to drugs, deep brain stimulation or pallidotomy are available. Stem cell therapy is also a promising innovation due to its ability to differentiate into nerve cells with specific functions.[5]

Parkinson's disease has a wide-ranging impact on the quality of life of sufferers and their families. Parkinson's revalence of 329 cases per 100,000 population with 16-19 new cases each year represents a significant health burden. The risk of the disease increases with age, with prevalence reaching 4% in populations over 80 years of age.[6] Despite the availability of various therapeutic options such as levodopa, dopamine agonists, and deep brain stimulation, current treatments are only symptomatic and have not been able to halt disease progression. [7] Oxidative stress and mitochondrial dysfunction have been identified as major mechanisms in the pathogenesis of Parkinson's disease. Exposure to environmental toxins such as rotenone can trigger both of these processes, causing free radical accumulation that damages dopaminergic nerve cells. This has prompted research to identify neuroprotective compounds that can protect neurons from oxidative damage.

[4] Suharti Suharti, "Patofisiologi Penurunan Kognitif pada Penyakit Parkinson," *UMI Medical Journal* 5, no. 1 (June 30, 2020): 1–11.

[5] Maria Diandra Porsiana and I Komang Arimbawa, "Terapi Stem Cell untuk Penyakit Parkinson" 47, no. 3 (2020).

[6] Safia Alia et al., "Penyakit Parkinson: Tinjauan Tentang Salah Satu Penyakit Neurodegeneratif yang Paling Umum," *AKSONA* 1, no. 2 (March 29, 2022): 95–99.

[7] Melvariani Syari Batubara, Emita Sabri, and Masitta Tanjung, "Andaliman (Zanthoxylum Acanthopodium Dc.) Terhadap Gambaran Morfologi Ovarium MENCIT (Mus musculus L.)" 1, no. 1 (n.d.).

Andaliman (Zanthoxylum acanthopodium DC) is a spice plant endemic to North Sumatra that has long been used in traditional medicine.[8,9] Previous studies revealed that andaliman extract contains various bioactive compounds such as alkaloids, flavonoids, saponins, and terpenoids that have antioxidant and anti-inflammatory activities.[10,11] Recent findings by Salim (2024) showed that andaliman ethyl acetate extract can protect dopaminergic cells from rotenone toxicity in experimental models.[12] Based on the identified neuroprotective potential of andaliman, this study aimed to test the hypothesis that administration of andaliman fruit extract can improve the survival of dopaminergic neurons in the substantia nigra in a rotenone-induced Parkinson's rat model. This study is the first to comprehensively evaluate the neuroprotective effect of andaliman extract against dopaminergic cell degeneration using an in vivo model. Wistar strain rats were chosen as the experimental model due to their metabolic similarity to humans, allowing for more relevant research results for clinical applications.[13,14]

THEORETICAL BASIS

Parkinson's disease is a neurodegenerative disorder that affects the central nervous system, specifically the extrapyramidal system that controls body

[8] dian Pratiwi Et Al., "Sebagai Tanaman Endemik Sumatera Utara" 31, no. 2 (2024).

[9] Nommensen Pangihutan Ompusunggu and Wahyu Irawati, "Andaliman (Zanthoxylum Acanthopodium DC.), a Rare Endemic Plant from North Sumatra that Rich in Essential Oils and Potentially as Antioxidant and Antibacterial," *Jurnal Biologi Tropis* 21, no. 3 (November 13, 2021): 1063–1072.

[10] Nurul Yasmin, Wahyu Widayat, and Angga Cipta Narsa, "Identifikasi Metabolit Sekunder Ekstrak Metanol Akar dan Batang Merung (Coptosapelta tomentosa) yang Memiliki Aktivitas Antioksidan Menggunakan Metode KLT Autografi," *Proceeding of Mulawarman Pharmaceuticals Conferences* 10 (October 31, 2019): 10–15.

[11] Ratih Anggraeni, "Uji Karakteristik Simplisia Buah Andaliman (Zanthoxylum acanthopodium DC.)," *JIFI (Jurnal Ilmiah Farmasi Imelda)* 3, no. 2 (March 28, 2020): 32–38.

[12] Reny Salim, "Phytochemicals & Antioxidant Activity of Andaliman Seeds and Flesh" (2024).

[13] David Pakaya and Rina Susilowati, "Pemanfaatan Hewan Coba Pada Penelitian Neurodegeratif" (n.d.).

movement.[14] The condition is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta, leading to decreased dopamine production. When dopamine levels decrease by 50%, patients begin to show typical symptoms such as tremors, muscle stiffness and difficulty moving. The disease affects not only the dopaminergic system but also other neurotransmitter systems such as glutamatergic, cholinergic, serotonergic, and adrenergic.[15] The clinical manifestations of Parkinson's can be divided into two main categories: motor and non-motor symptoms.[16] Motor symptoms include tremors that usually begin on one side of the body, bradykinesia (slowed movement), muscle stiffness, and impaired balance that increases the risk of falls.[17] Meanwhile, non-motor symptoms include flat facial expressions, excessive salivation, loss of smell, difficulty speaking and swallowing, and various neuropsychiatric disorders such as depression, anxiety, and dementia.[18]

One of the key factors in the development of Parkinson's is oxidative stress due to free radicals that damage nerve cells.[19] Although there are synthetic antioxidants such as BHA and BHT that are effective, their use is limited due to potential side effects.[20] This has prompted research into natural alternatives, one of which is andaliman fruit (Zanthoxylum acanthopodium). Andaliman contains bioactive compounds such as flavonoids and alkaloids that have strong

[14] Alia et al., "Penyakit Parkinson."

[15] Raudah Novita Putri and Intan Sahara Zein, "Parkinson Disease," *An-Najat* 1, no. 4 (October 25, 2023): 266–278.

[16] Francesca Magrinelli et al., "Pathophysiology of Motor Dysfunction in Parkinson's Disease as the Rationale for Drug Treatment and Rehabilitation," *Parkinson's Disease* 2016 (2016): 1–18.

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

[18] Shirley Yin-Yu Pang et al., "The Interplay of Aging, Genetics and Environmental Factors in the Pathogenesis of Parkinson's Disease," *Translational Neurodegeneration* 8, no. 1 (December 2019): 23.

[19] Priya Nugraha and Muhammad Hamdan, "Profil Gejala Motorik dan Non-Motorik pada Pasien Penyakit Parkinson di RSUD Dr. Soetomo Surabaya" 1 (n.d.).

[20] Magrinelli et al., "Pathophysiology of Motor Dysfunction in Parkinson's Disease as the Rationale for Drug Treatment and Rehabilitation."

antioxidant activity. Research shows that andaliman extract can protect dopaminergic cells from damage, reduce oxidative stress, and has anti-inflammatory properties that support nerve cell survival.[21,22] To study the effectiveness of andaliman in treating Parkinson's, researchers used an experimental model with rotenone.[23] Rotenone is a natural compound from tropical plants that can replicate Parkinson's symptoms in experimental animals.[24] It works by inhibiting mitochondrial complex I, resulting in impaired cellular energy production and increased free radicals.[25] The fat-soluble nature of rotenone allows it to penetrate the blood brain barrier and induce nerve cell damage similar to that in Parkinson's disease.[26,27]

Research using this rotenone model is important for understanding the mechanisms of Parkinson's pathogenesis and developing more effective treatment strategies.[28] The combination of understanding the neurotoxic effects of rotenone and the neuroprotective potential of andaliman opens up new

[21] Andre Lukas and Ismail Setyopranoto, "Korelasi antara ansietas, depresi, dan gangguan kognitif terhadap kualitas hidup penderita penyakit Parkinson" 17, no. 3 (2018).

[22] Rohmania Setiarini, "Hubungan Durasi Penyakit Dengan Gejala Non Motorik Penyakit Parkinson" (n.d.).

[23] Héctor Alberto González-Usigli et al., "Neurocognitive Psychiatric and Neuropsychological Alterations in Parkinson's Disease: A Basic and Clinical Approach," *Brain Sciences* 13, no. 3 (March 18, 2023): 508.

[24] Fitri Handajani, "Oksidan Dan Antioksidan Pada Beberapa Penyakit Dan Proses Penuaan" (n.d.).

[25] Ompusunggu and Irawati, "Andaliman (Zanthoxylum Acanthopodium DC.), a Rare Endemic Plant from North Sumatra that Rich in Essential Oils and Potentially as Antioxidant and Antibacterial."

[26] 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.

[27] Irvina M. Nuh Siagian et al., "Pengaruh Pemberian Ekstrak Daun Andaliman (Zanthoxylum Acanthopodium Dc.) Terhadap Jumlah Leukosit Total dan Leukosit Jenis Tikus Putih (Rattus Novergius L.) yang Diinduksi Boraks," *Jurnal Pendidikan Sains dan Komputer* 3, no. 01 (December 21, 2022): 17–30.

[28] Peiwen Zhang et al., "Plants in the Genus Tephrosia: Valuable Resources for Botanical Insecticides," *Insects* 11, no. 10 (October 21, 2020): 721.

opportunities in the development of therapies for Parkinson's disease.[29] Studies show that administration of andaliman extract can decrease the number of cells undergoing necrosis in experimental models, making it a promising candidate for the development of natural material-based Parkinson's therapies.[30,31]

RESEARCH METHODS

This study used a quantitative experimental approach with a completely randomized design (CRD). This design ensures that treatments are randomized to reduce bias, evaluate treatment effects, and compare results between groups objectively. The research was conducted at the Pharmacology Laboratory of the Faculty of Pharmacy, University of North Sumatra and the Anatomical Pathology Laboratory of Royal Prima Medan Hospital for more than 3 months from August to October 2024. The experimental animal research protocol was approved by the Prima Indonesia University Health Research Ethics Committee (040/KEPK/UNPRI/V/2024). This study used 70% ethanol solution, andaliman fruit, filter paper, ketamine, 70% alcohol, 80% alcohol, 90% alcohol, absolute alcohol I, absolute alcohol II, absolute alcohol III, xylol I, xylol II, xylol III, paraffin I, paraffin II, Hematoxylin-Eosin (HE), and Na.CMC 1%. While the tools used are rotary evaporator, scalpel, dropper, mask, gloves, paraffin block, tapered container, rotary vacuum, tissue processor, stationery, scales and markers.

This study was initiated by extracting andaliman fruit (*Zanthoxylum acanthopodium DC*) using the maceration method using 70% ethanol . A total of 1.5 kg of white temu was washed and sliced thinly then dried in the oven at 40 ^{o} for 1 week. The dried andaliman fruit was pulverized into powder, after which it was extracted using the maceration method accompanied by stirring until

[29] Pamela Jurdilla and Hary Sanjaya, "Kelarutan Zat Warna Organik dalam Gelasi Mikroemulsi Water In Oil Sistem Air, Surfaktan Kationik Hexadecyl Trimethyl Ammonium Bromide (HTAB) dan Pentanol" 11, no. 1 (2022).

[30] Ekramy Elmorsy et al., "Differential Effects of Paraquat, Rotenone, and MPTP on Cellular Bioenergetics of Undifferentiated and Differentiated Human Neuroblastoma Cells," *Brain Sciences* 13, no. 12 (December 14, 2023): 1717.

[31] I Dewa Ayu Susilawati, "Kajian Pustaka: Sumber Reactive Oxygen Species (ROS) Vaskular," *STOMATOGNATIC - Jurnal Kedokteran Gigi* 18, no. 1 (March 22, 2021): 1.

homogeneous. Maceration was continued by evaporation using a vacuum rotary evaporator to produce a solvent-free thick extract. After that, phytochemical screening testing of andaliman fruit extract was carried out to determine the type of biocative compounds contained in andaliman fruit extract.[32]

25 male mice were used in this study after an acclimatization period of 7 days.[33] Mice were divided into five groups, each consisting of five animals. The negative control group (K-) only received distilled water subcutaneously every other day for 28 days. The positive control group (K+) was given rotenone subcutaneously at a dose of 3 mg/kgBB every other day for 14 days. Treatment group 1 (P1) received the same rotenone induction for 14 days, followed by the administration of andaliman fruit extract as much as 150 mg/kgBB orally through a roundabout for 14 days. Treatment group 2 (P2) received similar treatment, but with a dose of andaliman extract of 300 mg/kgBB. Treatment group 3 (P3) was given the same treatment, but with an extract dose of 450 mg/kgBB. This study was conducted to observe the effect of andaliman fruit extract on the condition of rats that had been induced by rotenone.

Brain tissue collection was performed on day 29 by dislocating the neck of the rats, and removal of the brain soaked in physiological NaCl solution, then fixed in Bouin and 10% NBF for 48 hours. The brain was processed into paraffin blocks, sliced 4 micrometers thick, and hippocampal sections were stained with hematoxylin-eosin. The staining process began with deparaffinization using xylol, followed by gradual rehydration with alcohol, then washing with running water. Hematoxylin staining was done for 8 minutes, followed by eosin for 2 minutes, then dehydration again with alcohol and silol. The preparations were

[32] Ari Sri Windyaswari et al., "Profil fitokimia selada laut (Ulva lactuca) dan mikro alga filamen (Spirogyra sp) sebagai bahan alam bahari potensial dari perairan Indonesia," *Kartika : Jurnal Ilmiah Farmasi* 7, no. 2 (December 15, 2019): 88–101.

[33] yoga Purnama Haidir, Gusti Ayu Rai Saputri, And Dessy Hermawan, "Uji Efektivitas Kombinasi Umbi Bawang Dayak (Eleutherine Palmifolia (L.) Merr) Dan Daun Insulin (Tithonia Diversifolia) Terhadap Penurunan Kadar Glukosa Darah Pada Tikus Putih (Rattus Norvegicus) Diinduksi Na2edta" 5, no. 1 (2022).

covered with glass coverslips using adhesive, labeled, and observed under a binocular microscope at 400 times magnification in five field of view.[34]

Data analysis was tested with Normality using the Levene test to ensure normal sample distribution, and Homogeneity Test with Shapiro-Wilk to determine data homogeneity. If the data was normally distributed, One Way ANOVA analysis was conducted with a significance level of $p < 0.05$. If the data is not normal or not homogeneous, data transformation is performed. If after transformation the data is still not normal and homogeneous, the Kruskal-Wallis nonparametric test is used with the same level of significance. Meaningful analysis was followed by post hoc test to determine significant differences between groups.[35] The workflow of this research can be depicted through the following diagram.

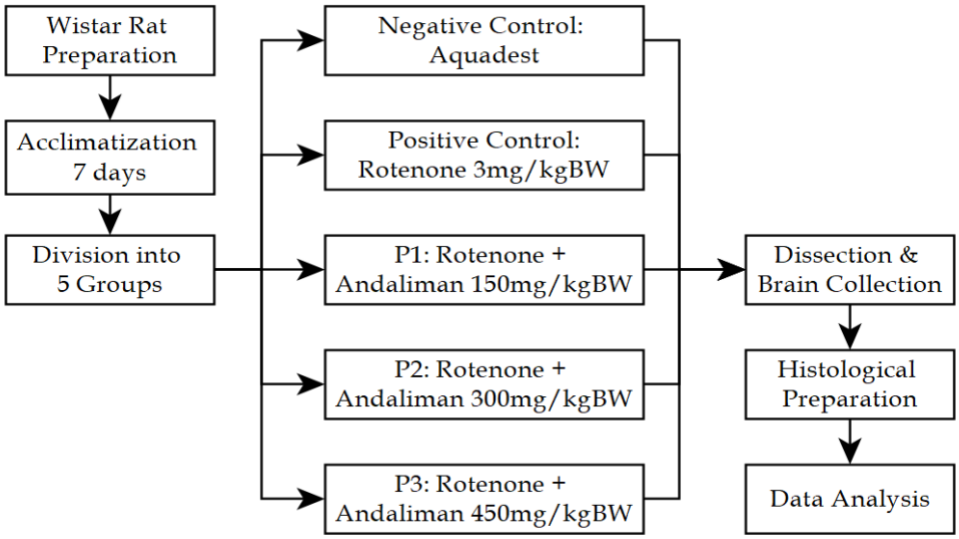


Figure 1. Research flow diagram

[34] Amelia, et al., "Gambaran Histopatologi Otak Mencit (Mus Musculus L) Setelah Pemberian Fraksi Daun Sembung Rambat (Mikania Micrantha Kunth) Sebagai Aktivitas Neuroprotektan," *Jurnal Farmamedika (Pharmamedica Journal)* 5, no. 1 (June 12, 2020): 30–37.

[35] Monikasari Monikasari et al., "Pengaruh pemberian ekstrak bekatul beras hitam (Oryza sativa L. indica) terhadap kadar MDA, SOD dan trigliserida pada tikus diabetes mellitus," *AcTion: Aceh Nutrition Journal* 8, no. 1 (March 25, 2023): 129.

RESULTS AND DISCUSSION

Results

1. Phytochemical content of ethanol extract of Andaliman (*Zanthoxylum Acanthopodium*)

Table 1. Phytochemical test of andaliman ethanol extract

Metabolic Content	Reagents	Ethanol Extract of Andaliman	Description
Alkaloids	Mayer	+	White precipitate formed
	Wagner	+	Brown precipitate formed
	Dragendorff	+	Forms a red precipitate
Steroids	Liebermann-Burchard Test	+	Green color formed
Terpenoids	Liebermann-Burchard Test	+	Formed red color
Saponins	Shuffling	+	Foaming
Flavonoids	HCL and Mg Metal	+	Purple color formed
Phenolics	FeCL3	+	Blue color formed
Tannins	Gelatin + H2SO4	+	White precipitate formed

Phytochemical screening of ethanol extract of andaliman fruit showed alkaloid, steroid, terpenoid, saponin, flavonoid, phenolic, and tannin contents. Alkaloid tests with Mayer, Wagner, and Dragendorff produced white, brown, and red precipitates. Steroid and terpenoid tests with Liebermann-Burchard reagent produce green and red colors. Saponin test produces foam, flavonoid test shows purple color, phenolic test produces blue color, and tannin test forms white precipitate.

2. Effectiveness of andaliman as an anti-oxidant on brain cells

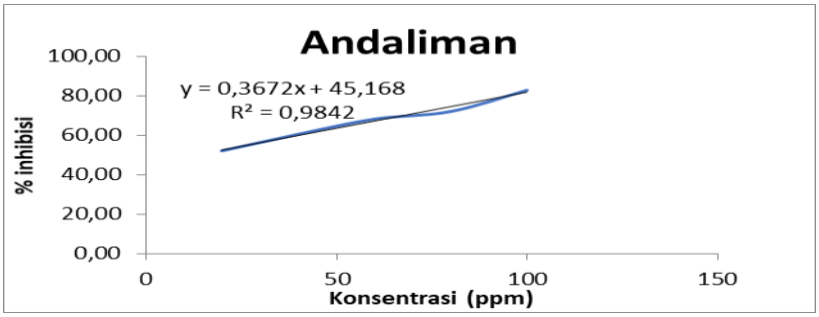


Figure 2. Curve of andaliman concentration with percentage inhibition

When the concentration of andaliman was reduced to 40 ppm and 20 ppm, the absorbance increased again with values of 0.352 and 0.428 respectively. As a result, the percentage of inhibition was lower, at 60.54% and 52.06%. This data indicates an inverse relationship between andaliman concentration and inhibition effectiveness, where inhibition decreases with decreasing concentration.

3. Observation of brain cells for 28 days

Table 2: Observation results of brain cells for 28 days

Group	Brain Cells					P-value
	1	2	3	4	5	
K-	0,4+0,54	0,4+0,54	0,2+0,44	0,2+0,44	0,4+0,54	,006
K+	4,8+0,44	4,8+0,83	5,0+0,70	4,8+0,83	4,6+0,89	,325
P1	3,4+0,54	4,0+0,70	4,4+1,14	4,8+0,83	4,4+1,14	,679
P2	4,6+1,51	3,6+0,89	3,0+0,70	3,4+0,54	2,8+0,83	,492
P3	1,8+0,83	1,4+0,54	1,0+0,70	1,2+0,44	1,2+0,44	,492

The analysis showed that the Negative Control Group (K-) had a significantly lower number of brain cells than the other groups with a P-value of 0.006. Meanwhile, the Positive Control Group (K+) and the three treatment groups (P1, P2, and P3) showed no significant difference, with a P-value greater than 0.05. Treatment Group 3 (P3) had the lowest number of brain cells, but still not statistically significant.

Table 3. Oneway Anova Test

Group	Brain Cells			
	Mean	SD	F	P
K-	0,32	0,10	102,96	0,00
K+	4,80	0,14		
P1	4,20	0,52		
P2	3,48	0,70		
P3	1,32	0,30		

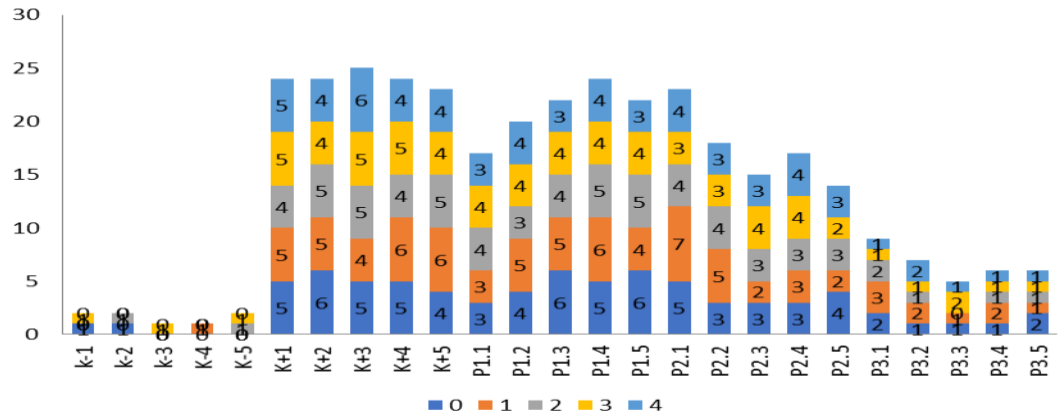
The results of the One-way ANOVA test in Table 3 show that the Negative Control Group (K-) had a very low mean number of brain cells (0.32 ± 0.10), with F = 102.96 and P-value = 0.00, which indicates a highly significant difference. The Positive Control (K+) group had a mean of 4.80 ± 0.14, while treatment groups P1, P2, and P3 had means of 4.20 ± 0.52, 3.48 ± 0.70, and 1.32 ± 0.30, respectively. A significant difference was seen between the negative control group and the treatment groups, with the P3 group showing the lowest brain cell count.

Table 4. *LSD post hoc* test

Comparison	**MD**	**P**
KN vs KP	-4,48	0
KN vs P1	-3,88	0
KN vs P2	-3,16	0
KN vs P3	-1	0,001
KP vs P1	0,6	0,037
KP vs P2	1,32	0
KP vs P3	3,48	0
P1 vs P2	0,72	0,014
P1 vs P3	2,88	0
P2 vs P3	2,16	0

LSD post hoc test results showed significant comparisons between the tested groups. The most significant difference was found between the Negative Control (KN) group and all other groups, with a P-value of 0.000 for KN vs KP, KN vs P1, KN vs P2, and 0.001 for KN vs P3, respectively. In addition, the comparison between the Positive Control group (KP) and treatment groups P1, P2, and P3 also showed significant differences, with P-values of 0.037, 0.000, and 0.000, respectively. The P1 vs P2 group (P-value 0.014) as well as P1 vs P3 and P2 vs P3 (P-value 0.000 respectively) also showed significant differences.

4. Scoring of morphological changes in brain cells



Scoring of morphological changes in brain cells

Cell necrosis scoring is used to assess the extent of cell damage in studies, especially those dealing with the effects of neurotoxic or other interventions. This method is generally performed by visually observing cell morphology, with criteria divided into several levels. A score of 0 indicates cells that do not undergo necrosis, with normal cell structure and intact cell nuclei. Score 1 indicates slight morphological changes, such as swelling or discoloration, with less than 25% of the cell area affected by necrosis. Score 2 indicates further damage, with swelling and organelle disintegration affecting 25% to 50% of the cell area. A score of 3 indicates more than 50% of the cell area has necrosis, with inflammatory cell infiltration and dead tissue visible. Finally, a score of 4 indicates complete necrosis of the entire area analyzed, with loss of tissue structure and no visible cell remnants.

DISCUSSION

Phytochemical test results on ethanol extract of andaliman (*Zanthoxylum Acanthopodium*) showed bioactive compounds, such as alkaloids, steroids, terpenoids, saponins, flavonoids, phenolics, and tannins. Alkaloids were detected with Mayer, Wagner, and Dragendorff reagents, which showed antimicrobial and neuroprotective potential. Steroids and terpenoids were also detected, both of which have anti-inflammatory and antioxidant potential.

Saponins that produce foam can enhance endurance, while flavonoids, which were detected with HCl and magnesium metal, function as antioxidants and antidiabetics. Phenolics and tannins showed the ability to capture free radicals and antibacterial properties. Overall, the phytochemical content of andaliman extract supports therapeutic potential, including antioxidant, antimicrobial, and antidiabetic.

The results of the study according to Salim,. 2024 is in line with previous findings showing that 70% ethanol extract from andaliman fruit (Zanthoxylum Acanthopodium) contains bioactive compounds such as alkaloids, steroids, terpenoids, saponins, flavonoids, phenolics, and tannins. Flavonoids and phenolics in andaliman fruit are known to have anti-infective, antidiabetic, and oxidative stress-fighting activities in humans. These antioxidant compounds function to protect the body from free radical damage by giving electrons to free radical molecules, thus inhibiting oxidation reactions in the body.[36] This study also found alkaloid, steroid, and tannin content in 70% ethanol extract of andaliman fruit. These findings are in line with the research of Kesavan et al (2027) who identified differences in phytochemical content in ethanol and water extracts of Zanthoxylum Acanthopodium. The phytochemical screening of the water extract in that study showed the presence of saponins and alkaloids, while the ethanol extract was richer in terpenoids and glycosides, but did not contain alkaloids. This shows that the type of solvent can affect the phytochemical content produced. In addition, factors such as growing location, rainfall, humidity, nutrients, temperature, and soil quality also affect secondary metabolites in simplisia. Another study conducted by Usliana, 2021 also reported phytochemical content including flavonoids, phenolics, alkaloids, terpenoids, saponins, steroids, and tannins in Zanthoxylum Acanthopodium.

Based on the inhibition analysis of compounds from andaliman in this study, we obtained the result that the concentration of 100 ppm showed the highest antioxidant activity with an inhibition percentage of 82.96%, which can inhibit free radicals. Similar results were also found in the research of Winarti et

[36] Salim, "Phytochemicals & Antioxidant Activity of Andaliman Seeds and Flesh."

al., 2018, where andaliman fruit extract at a concentration of 150 ppm was able to inhibit free radical activity by more than 70%, that the highest antioxidant activity in ethanol extract of andaliman fruit occurred at a concentration of 200 ppm, with an inhibition value of 61.81%.[37]

The results of One Way Anova test in this study also showed that the administration of andaliman extract can increase the number of rotenone-induced brain cells. Group P1 (dose of 150 mg/kgBB) showed results close to the positive control, indicating that this dose is optimal in increasing antioxidant activity on the number of damaged brain cells. In contrast, higher doses in the P3 group showed a decrease in the number of brain cell necrosis, indicating potential toxicity or reduced effectiveness at higher doses ($p<0.00$). This finding is in line with the study of Hermayeni et al., 2022, which showed that andaliman fruit extract has antioxidant activity against cancer cells in benzopyrene-induced cancer model rats ($p<0.01$).[38] Similar results were also found in the study of Tarigan et al., 2024 which reported that andaliman ethanol extract can repair benzopyrene-induced liver and kidney cell damage ($p<0.01$).[39]

Histopathological observations show that brain cells are damaged, in the form of necrosis or cell death, due to exposure to nephrotoxic substances. According to Underwood, 2020 necrosis can be caused by toxins, viruses, or lack of oxygen, which leads to cell or tissue death. Morphological changes observed in dead cells include cell nucleus shrinkage, shriveled chromatin, and cells that become eosinophilic (karyolysis). Treatment with andaliman fruit extract, both low and high doses, showed a positive development, with brain cells returning

[37] Wiwi Winarti, Partomuan Simanjuntak, and Muhammad Fahmi Syahidin, "Identifikasi Senyawa Kimia Aktif Antioksidan Dari Ekstrak Etil Asetat Buah Andaliman (Zanthoxylum acanthopodium DC)," *Talenta Conference Series: Tropical Medicine (TM)* 1, no. 3 (December 20, 2018): 162–166.

[38] Rostime Hermayerni et al., "Cytochrome c Expression by Andaliman (Zanthoxylum Acanthopodium) on Cervical Cancer Histology," *Pakistan Journal of Biological Sciences* 25, no. 1 (December 15, 2021): 49–55.

[39] Novriani Tarigan, Jansen Silalahi, and Urbanus Sihotang, "Formulation of Recipes and Organoleptic Properties of Tinuktuk as Simalungun Traditional Food," *Jurnal Health Sains* 5, no. 2 (February 22, 2024): 85–94.

to normal. This is in line with the research of eka et al., 2012 which found changes in cell morphology in hepatotoxicity due to chemical compounds, which were also improved by the administration of andaliman fruit extract.

CONCLUSION

Based on the results of the research that has been done, 70% ethanol extract of Andaliman fruit (Zanthoxylum Acanthopodium) shows effectiveness in terms of cell repair characterized by a decrease in the number of necrosis cells in hitopathology. 70% ethanol extract of Andaliman fruit (Zanthoxylum Acanthopodium) was able to show antioxidative and neuroprotective functions on necrosis cells with the most effective dose of 450 mg/kgBB based on the number of normal cell regeneration. There was a statistically significant increase in the administration of Andaliman fruit extract with a positive effect on the number of rotenone-induced brain cells with a value of ($p<0.05$). This finding supports the hypothesis that the intervention of 70% ethanol extract of Andaliman fruit (Zanthoxylum Acanthopodium) has a positive effect on brain cell damage in substantia nigra against Parkinson's disease.

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