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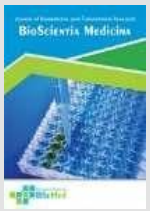

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Antidiabetic Effectiveness Test of Andaliman Fruit (*Zanthoxylum acanthopodium*) Extract on Histopathological Changes in Peripheral Nerves: An In Vivo Study

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A B S T R A C T

Background: Diabetes mellitus (DM) is a chronic metabolic disease characterized by elevated blood sugar levels, and the increase in sugar levels is triggered by low insulin production or the ineffective use of insulin by the body (relatively). Diabetic neuropathy is a complication that often attacks DM patients and is the highest complication of diabetes. This study aims to test the effectiveness of Andaliman fruit (*Zanthoxylum acanthopodium*) extract on the improved histopathology of Peripheral nerve in alloxan-induced Wistar rats (*Rattus norvegicus*). **Methods**: This study is an experimental study using 24 Wistar rats, which were grouped into 6 groups (1: normal, 2: negative control, 3: positive control, 4-6: DM + andaliman extract 150mg/kgBW, 250 mg/kgBW, 350 mg /kgBB). Data analysis used SPSS to determine differences in the histological conditions of peripheral nerves. **Results:** The administration of andaliman fruit extract at a dose of 350 mg/kgBW was most effective in inhibiting the proliferation of Schwann cells, which showed the potential of andaliman fruit extract in preventing the progression of diabetic neuropathy. The administration of andaliman fruit extract at a dose of 350 mg/kgBW also showed the most effective potential in inhibiting the inflammatory process in the peripheral nerves, which is believed to be responsible for peripheral nerve cell damage. **Conclusion:** Andaliman fruit (*Zanthoxylum acanthopodium*) extract is effective against improving the histopathology of peripheral nerves in alloxane-induced Wistar rats (*Rattus norvegicus*).

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by elevated blood sugar levels, and the increase in sugar levels is triggered by low insulin production or ineffective use of insulin by the body (relatively).[1,2] Patients with diabetes are indicated by the concentration of glucose in the blood that exceeds the normal limit, which in the end, all the glucose that has been filtered out cannot be reabsorbed by the kidneys, and this leads to the appearance of glucosuria. In addition, there will also be complaints of polyuria, polydipsia, and weight loss triggered by disruption of protein metabolism, in which excessive protein circulating in the blood cannot be stored in the tissues and is caused by increased fat metabolism.[3] Diabetic neuropathy is a complication that often affects DM patients and is the highest complication of diabetes in developing countries and has the status as a trigger that causes 50% to 75% of non-traumatic amputations. The highest complication in the range of 60% is peripheral nerve dysfunction which is generally termed diabetic neuropathy.[4]

Long-term use of antidiabetic drugs and the use of insulin injections can pose a dangerous risk of side effects caused by drugs. Therefore, it is found that many people with DM have switched to using herbal medicines for their alternative therapy. Andaliman

plant (*Zanthoxylum acanthopodium DC*) is categorized as a spice plant as well as an essential oil producer, which is commonly found in North Sumatra, especially in areas with an altitude of 1,500 meters above sea level.[5] Apart from being a spice, Andaliman is also used as an optional treatment, for example, as an aromatic ingredient, tonic, appetite stimulant, and medicine to relieve stomach pain and diarrhea.[6] The activity of ethanol extract from Andaliman fruit as an anti-inflammatory carried out in vitro has also been shown to be able to counteract tumor necrosis factor, interleukin, and cyclooxygenase in the inflammatory process.[7] In carrying out the phytochemical examination, several compounds were identified in Andaliman, for example, alkaloids, flavonoids, triterpenoids, steroids, and saponins. In addition, flavonoids are believed to have inhibitory activity against the a-glucosidase enzyme, and DM disease can be affected by the activity of these enzymes and antioxidants.[8] This study aimed to test the effectiveness of Andaliman fruit extract *(Zanthoxylum acanthopodium)* on the improvement of Peripheral nerve histopathology in alloxan-induced Wistar rats (*Rattus norvegicus).*

2. Methods

This study is an experimental study with a post-test-only approach with a control group. A total of 24 male rats (*Rattus norvegicus*) Wistar strain, bodyweight 150-210 grams and age 8-12 weeks. After acclimatization for 7th days, the Rats were grouped into 6 groups, where each group consisted of 4 Rats. Group 1: Normal control rat group, Group 2: Negative control group given alloxan 90mg/Kg BW, Group 3: A group of rats given alloxan, then continued with Glibenclamide 10mg/kgBW, Group 4: A group of rats given alloxan, which was continued by giving the ethanol extract of andaliman fruit as much as 150 mg/Kg BW, Group 5: The group of rats given alloxan, followed by giving the ethanol extract of the andaliman fruit 250 mg/Kg BW, Group 6: The group of rats given alloxan, and continued by giving andaliman fruit ethanol extract 350 mg/Kg BW. This study has been approved by the medical and health research ethics committee of the Faculty of Medicine, Universitas Prima Indonesia.

A total of 3 kg of andaliman fruit samples that have been dried and mashed were extracted using the maceration method. Maceration was carried out with 96% ethanol solvent for 3x24 hours. The obtained macerate is then evaporated to obtain a thick extract. After the observation period ended, the White Rat was euthanized using 10% chloral hydrate. Peripheral nerve evacuation was performed in the tibial region. The evacuated neural tissue was then put into a 10% neutral buffered formalin (NBF) solution. Then the paraffinization process was carried out to obtain tissue paraffin blocks and cut with a thickness of 5 micrometers. The tissue paraffin block sections were then subjected to the HE staining process. Further observations were made to determine the condition of the peripheral nerves. Data analysis was carried out with the help of SPSS version 26 software. Furthermore, univariate analysis was carried out to determine the frequency distribution of each test variable. Bivariate analysis was performed to determine differences between test groups with Repeated ANOVA and Post hoc LSD, with a significance of $p<0.05$.

3. Results

Table 1 shows the data that before induction, there was a comparison of KGD in the negative group (76.50 ± 6.56) and the extract dose of 350 mg/kgBB (153.50 ± 158.49) found a significant difference, while on the other hand, in the normal group, glibenclamide at a dose of 0.45 mg/kgBW, extract at a dose of 150 mg/kgBW and 250 mg/kgBW did not find a significant difference. On the 3rd day, after induction, comparison of KGD between the normal group and each treatment group. And after the 7th day of treatment, there was a significant decrease in KGD with Glibenclamide (232.25 ± 40.26), extract dose of 150 mg/kgBW (306.50 ± 46.51), extract dose 250 mg/kgBW (292 .75 ± 71.91) and extract dose of 350 mg/kgBW (268.25 ± 34.86) were compared with the negative group (408.00

± 32.71). And after 14th days of treatment, there was a significant decrease in KGD in the group that received glibenclamide 0.45 mg/kgBW, extract dose 150 mg/kgBW, 250 mg/kgBW and 350 mg/kgBW compared to the negative control, but the decrease in KGD did not reach the normal limit. From the results of the paired difference test, it was found that in the normal group and the Andaliman extract at a dose of 350 mg/kgBW there was a significant difference, while in the negative group, the drug glibenclamide was 0.45 mg/kgBW, at a dose of 150 mg/kgBW and at a dose of 250 mg/kgBW, there was no significant difference. From the results of the unpaired difference test on initial blood glucose levels, significant and homogeneous differences were found. Then on the 3rd day, there were no significant differences in glucose levels, then significant differences were found on the 7th day, and significant differences were identified and homogeneous on the 14th day.

Table 1. Comparison of blood sugar levels (KGD) of Wistar rats between groups.

Groups	KGD				p
	Early	3rd Day	7th Day	14th Day	
Normal	83,75 ± 6,60	84,25 ± 4,03	84,00 ± 5,23	86,00 ± 6,06	0,552†
Negative	76,50 ± 6,56	368,50 ± 45,49	408,00 ± 32,71	448,75 ± 85,11	0,038†*
Glibenclamide 0,45	76,75 ± 8,30	363,50 ± 62,19	232,25 ± 40,26	117,50 ± 18,01	0,185¶
Extract 150	87,00 ± 13,74	381,75 ± 76,21	306,50 ± 46,51	251,25 ± 34,04	0,099¶
Extract 250	83,75 ± 14,91	406,50 ± 95,01	292,75 ± 71,91	206,50 ± 35,24	0,306¶
Extract 350	153,50 ± 158,49	442,50 ± 93,92	268,25 ± 34,86	163,00 ± 12,52	0,026†*
P	0,658‡	0,038‡*	<0,001§*	0,001‡*	

* Significant, p < 0.05; § One Way ANOVA; ‡ Kruskal Wallis; ¶ Repeated ANOVA; † Friedman.

Table 2. Histological comparison of peripheral nervous tissue between groups.

Variables	Group	Mean±SD
Schwann Cells	Normal	30±7.6
	Negative	104.5±35.1
	Glibenclamide drug	61.6±22.9*
	Dosage 150 mg/kgBW	50.8±23.6*
	Dosage 250 mg/kgBW	86.1±33.4*
	Dosage 350 mg/kgBW	35.4±8.9*
Inflammation	Normal	0
	Negative	1±0.0
	Glibenclamide drug	0*
	Dosage 150 mg/kgBW	0.58±0.3*
	Dosage 250 mg/ kgBW	0.08±0.1*
	Dosage 350 mg/kgBW	0*

*Post hoc LSD VS Negative group, p<0.05

Table 2 shows an assessment of the histological aspects of peripheral nerves, as indicated by the mean number of Schwann cells and the presence of inflammation. The administration of andaliman fruit extract was able to reduce the average number of Schwann cells compared to the negative control group, which was only induced by DM. The administration of andaliman fruit extract at a dose of 350 mg/kgBW was most effective in inhibiting the proliferation of Schwann cells, which showed the potential of andaliman fruit extract in preventing the progression of diabetic neuropathy. The administration of andaliman fruit extract at a dose of 350 mg/kgBW also showed the most effective potential in inhibiting the inflammatory process in the peripheral nerves, which is believed to be responsible for peripheral nerve cell damage.

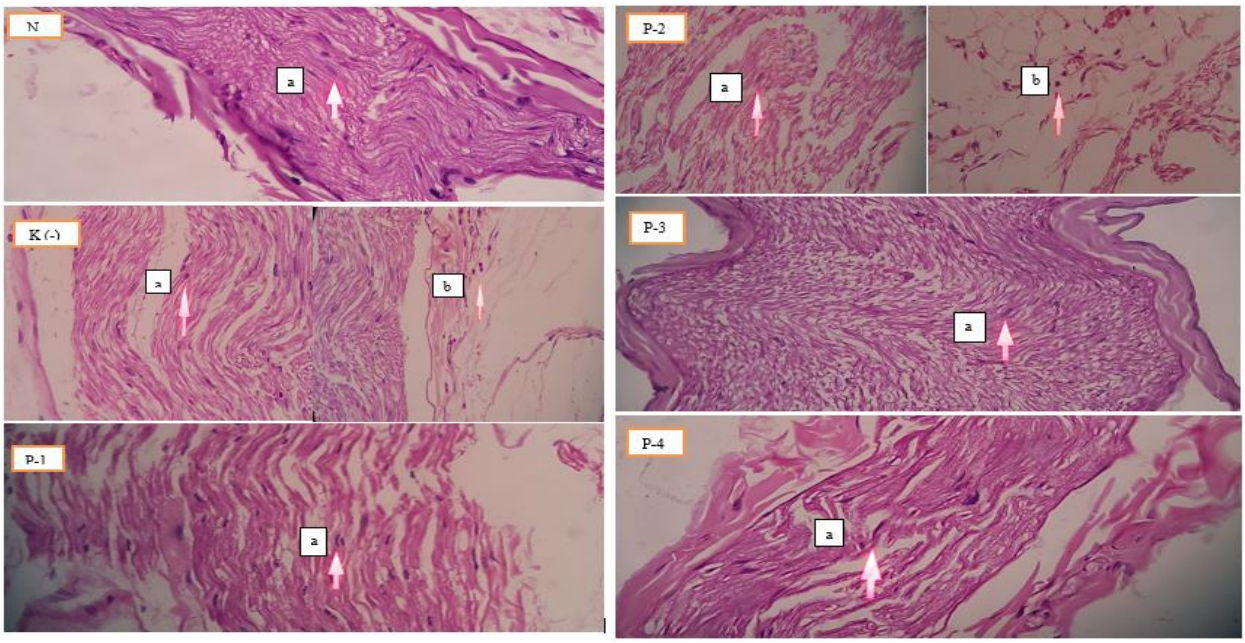

Figure 1. Histological appearance of peripheral nerve cells, magnification 400 x. Control Group (N), Negative Group (K (-)), and Glibenclamide Drug Group (P-1) Dosage group 150 mg/kgBW (P-2), Dosage group 250 mg/kgBW (P-3), and Group Dosage 350 mg/kgBW (P-4). White arrows indicate peripheral nerve cells.

4. Discussion

The structure of the peripheral nerve fibers is categorized as the basic functional unit of the nervous system. The metabolic ability of neurons is really high, but they cannot store nutrients or oxygen. Therefore, neuroglia are needed that can support neurons so that nutrients and oxygen can be supplied so that their survival is maintained. There are a number of supporting cells that have an essential role, including satellite cells and Schwann cells. The properties of Schwann cells in the peripheral nervous system are similar to oligodendroglia in the central nervous system. However, in terms of the formation of new cells, Schwann cells have nothing in common with oligodendria. If there is damage that occurs in the peripheral nerves, Schwann cells will give rise to a series of cylinders that have a crucial role in showing the direction of growth of the axon.[9-14]

Previous studies have shown antidiabetic activity by repairing kidney cells that were damaged by the discovery of relatively high blood glucose levels. Prolonged hyperglycemia results in diabetic neuropathy, which ultimately increases the activity level of the polyol pathway, synthesis of advanced glycation end products (AGEs), formation of free radicals, and activation of protein kinase C (PKC). These activated pathways will have effects, including lack of vasodilation, which in turn triggers a decrease in blood flow to nerves, then along with low myoinositol in cells, eventually diabetic neuropathy arises.[15]

Diabetic neuropathy is divided into three systems based on the anatomy of the peripheral nerve fibers, namely the motor system, sensory system, and autonomic system. Where the clinical manifestations vary and are really influenced by the type of nerve fiber lesion, peripheral nerves will be damaged due to diabetes mellitus in the form of nerve tissue that does not require insulin in terms of glucose transport, and vice versa, which is used as an alternative metabolic polyol pathway for glucose metabolism. Glucose is converted to sorbitol, where sorbitol is relatively slow to be converted to fructose. Glucose that accumulates from chronic hyperglycemia and then combined with a relatively slow conversion rate from sorbitol to fructose will eventually lead to sorbitol that accumulates in

peripheral nerves. The increase in sorbitol can disrupt the ion pump, namely by producing osmotic pressure by the image in the fluid, which ultimately reduces nitric oxide, increases reactive molecular oxygen, and triggers an increase in oxidative stress levels. These elements trigger the destruction of Schwann cells and ultimately disrupt nerve conduction. PKC activation is considered inappropriate as a result of hyperglycemia, which may also contribute to the development of neurological complications. PKC is an intracellular signaling molecule that regulates vascular function, the level of which is elevated in diabetes.

The activation of PKC in the nerves of blood vessels will lead to the emergence of vascular damage, and nerve conduction will decrease. The result of the binding of glucose metabolites to proteins is the end result of AGEs. Despite its status as a normal element of protein, the basement membrane in smaller blood vessels and uncontrolled blood glucose levels will support the over-production of AGEs. The increase in AGEs triggers the thickening of the basement membrane, which contributes to a decreased oxygen supply because neuronal dysfunction is strongly associated with vascular disorders, including AGE-induced nerve damage. Additional microvascular damage includes protein trapping (including LDL), inactivation of nitric oxide, and diminished vasodilation due to accumulation of the sorbitol and polyol pathways, activation of protein C kinase, and excessive accumulation of AGEs, all of which predispose to nerve damage via myelin degeneration, leading to loss of the ability of nerves to transmit signals. The onset of peripheral neuropathy is caused or triggered by fairly serious nerve damage, which in the end, causes a reduction or absence of nerve transmission by raising the possibility of the appearance of symptoms, including tingling, pain, and numbness.[16-20]

5. Conclusion

Andaliman fruit *(Zanthoxylum acanthopodium)* extract is effective in improving the histopathology of peripheral nerves in alloxane-induced Wistar rats (*Rattus norvegicus*).

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