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Neuroprotector Effects of Moringa Leaf (Moringa Oleifera Lamk) Ethanol Extract On Type Two Diabetes Rats Induced By Streptozotocin-Nicotinamid

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Ringkasan: Daun kelor (Moringa Oleifera Lamk) telah banyak digunakan sebagai terapi alternatif penyakit diabetes melitus, didukung oleh bukti ilmiah. Namun, pengaruh daun kelor terhadap neuropati diabetik belum diteliti. Penelitian ini bertujuan untuk mengetahui waktu terjadinya neuropati diabetik dan aktivitas neuroprotektif ekstrak daun kelor pada induksi streptozotocin-nicotinamide (STZ-NA). STZ-NA diinduksi secara intra peritoneal selama tiga hari untuk mendapatkan kondisi hiperglikemik pada tikus, sedangkan pengurangan jumlah makanan pada hewan uji dilakukan untuk mendapatkan defisiensi nutrisi yang akan mempercepat terjadinya neuropati diabetik. Injeksi intra peritoneal 65 mg/kg bb STZ dan 150 mg/kg bb NA dilakukan dan diamati sampai terjadi neuropati diabetik. Ekstrak etanol daun kelor diberikan secara oral setelah hewan uji mengalami neuropati diabetik pada hari ke 28 dengan dosis 50.100 dan 150 mg/kg bb. Glibenclamide, Vitamin B6, Glibenclamide + vitamin B6 digunakan sebagai obat kontrol. Akibatnya, kekurangan nutrisi dapat mempercepat terjadinya neuropati diabetik. Ekstrak etanol daun kelor dosis 100, 150 mg/kg bb memberikan aktivitas lebih baik dibandingkan glibenklamid, namun tidak lebih baik dibandingkan glibenklamid+vitamin B6. Neuropati diabetik pada tikus malnutrisi terjadi pada hari ke 28 setelah induksi STZ-NA. Dosis ekstrak etanol daun kelor yang paling ampuh untuk dikembangkan sebagai neuroprotektor adalah dosis 150 mg/kg bb.

Abstrack : *Moringa leaf (Moringa Oleifera Lamk) has been widely used as an alternative therapy for diabetes mellitus, supported by scientific evidence. However, the effect of moringa leaves on diabetic neuropathy has not been studied. This study aims to determine the timing of the occurrence of diabetic neuropathy and the neuroprotective activity of moringa leaf extract on streptozotocin-nicotinamide (STZ-NA) induction. STZ-NA was induced intra peritoneal for three days to obtain hyperglycemic conditions in rats, while reducing the amount of foods in test animals was carried out to get the nutritional deficiencies that will accelerate the occurrence of diabetic neuropathy. Intra peritoneal injection of 65 mg/kg bb STZ and 150 mg/kg bb NA were performed and observed until diabetic neuropathy occurred. Extract ethanol of Moringa leaf was given orally after the test animals had diabetic neuropathy on day 28 with doses of 50,100 and 150 mg/kg bb. Glibenclamide, Vitamin B6, Glibenclamide + vitamin B6 were used as medicines control. As result, lack of nutrition can accelerate the occurrence of diabetic neuropathy. Moringa leaf ethanol extract dose of 100, 150 mg/kg bb gave better activity than glibenclamide, but it's not better than glibenclamide+vitamin B6. Diabetic neuropathy in malnourished rats occurred on day 28 after STZ-NA induction. The most potent dose of ethanol extract of moringa leaves to be developed as a neuroprotector is a dose of 150 mg/kg bb.*

INTRODUCTION

Diabetes mellitus is a condition that affects the body's ability to properly use glucose (a type of sugar) for energy. It is a chronic disorder that can lead to various complications in different body systems. These complications can be classified as either short-term or long-term. Short-term complications include hypoglycemia (low blood sugar) and ketoacidosis (a condition where the body produces high levels of ketones). Long-term complications can include damage to large blood vessels (macroangiopathy) and small blood vessels (microangiopathy) ("Acute and Chronic Complications," 2017; Bjerg et al., 2021; Harding et al., 2019).

Nerve damage due to metabolic disorders of blood sugar levels is called diabetic neuropathy. As many as 60% of diabetic sufferers got complications of diabetic neuropathy (ND), and the prevalence of neuropathy in DM patients is estimated at 8% for new patients diagnosed with DM more than 50% in patients who have long been diagnosed with DM (Harding et al., 2019). Neuropathy Peripheral is one of the complications that often occurs in diabetes, causing pain, skin ulcers, muscle weakness, and decreased overall quality of life of patients (Yapanis et al., 2022). Diabetic neuropathic pain (NND) is one of the complications that often occurs, and it's most often complained of due to damage or dysfunction of the peripheral nerves caused by DM.

The flavonoids that are contained in Moringa Oleifera can work as insulin secretagogues or insulin mimetics, which ultimately minimizes diabetes complications. The research on phytochemical compounds in Moringa plants shows that the bioflavonoid compounds contained therein also have a role in stimulating glucose uptake in peripheral tissues so that they can reduce blood glucose (Haber et al., 2020; Vargas-Sánchez et al., 2019).

It is not known exactly when the appearance of neuropathic disorders occurs. This is due to many influencing factors, including metabolism in the body, such as diabetes and kidney disease and another thing that affects the timing of the appearance of neuropathic disorders is nutritional nutrition, which includes B1, B6, B12 and folic acid deficiencies (Castelli et al., 2020). Toxic substances such as metals and medicines also affect the length of time the onset of neuropathic disorders (Colvin & Dougherty, 2015).

This study was expected to be a reference to overcoming various complications due to DM conditions, to determine the length of time for symptoms of neuropathy complications in DM conditions, neuroprotector activity of Moringa leaf ethanol extract (Moringa oleifera Lamk.) in streptozotozine (STZ)-Nicotinamide (NA) induced rats). The research on ethanol extract of Moringa leaves to obtain the most potent activity in improving the speed of sensory nerve responses to thermal stimulation.

METODE

This research was carried out in two steps, the first step was optimizing the timing of the occurrence of diabetic neuropathy and the second step was the extract test. The optimization is to find out the exact timing of the occurrence of diabetic neuropathy is marked by a decrease in the ability of the nerves to respond to heat stimuli, while the extract test is to determine the neuroprotector activity of the ethanol extract of Moringa leaves.

Moringa leaves were obtained from Kupang, Alak District, Kupang City, East Nusa Tenggara. Moringa leaves are washed and dried by aerating before being made into powder. Moringa leaf powder was macerated in 96% ethanol for 5 days. The extract was thickened using an evaporator before being used. Identification of alkaloids and flavonoids was carried out by thin layer chromatography, based on standard procedures.

All trial protocols were approved by the Health Research Ethics Committee, Faculty of Medicine, Sultan Agung Islamic University, Semarang (Letter Number 237/VII/2022/Bioethics Commission).

Animal

Seventy-two males Wistar albino rats were used in this study. Rats were acclimatized for 1 week. Prior to STZ-NA induction, blood sugar levels were measured to determine the success of induction. NA (150 mg/kg bb) was injected intraperitoneally 15 minutes before STZ intraperitoneal injection (65 mg/kg bb). Blood sugar levels were measured 3 days after STZ-NA induction, rats with glucose levels above 200 mg/dl were declared diabetic. Four groups of rats, two of which were fed with a standard amount (12 grams per day) while the other two groups were fed a reduced amount (6 grams per day). Blood sugar levels and body weight were measured every 7 days, namely on days 7,14,21 and 28. In addition, the ability to respond to thermal stimuli was also continuously observed every 7 days using the tail flick and hot plate methods (on day 7th, 14th, 21st, 28th). The results showed that the group of rats with insufficient food had neurotoxicity on day 28th, while the group of rats with standard food had neurotoxicity on day 28th.

The first group of rats were given standard foods with the amount of 12 grams per day, and then only induced using 1% CMC. The second group of rats were given standard foods with a reduced amount of 6 grams per day. Reducing the amount of food given is intended so that the rats lack nutritional deficiencies. Furthermore, rats were also induced by 1% CMC. The third group of rats was induced by STZ-Na, but the food given was standard with the amount of 12 grams per day. And rats in group 4 were induced by STZ-Na, the amount of food given was 6 grams per day. The same test was carried out on 4 groups of rats, to find out when the neurotoxicity occurred, which was characterized by a decrease in sensory nerve activity. The group of rats that had the fastest decrease in activity was declared as the fastest group to obtain the condition of neuropathic rats, which was supported by the results of statistical data processing. The results showed that the rats of group four had neurotoxicity faster than the rats of group 3. This indicates that malnutrition can accelerate the appearance of diabetic neuropathy.

The next test was to see the neuroprotective activity of the ethanol extract of Moringa leaves. In this study, 40 Wistar rats were used with an average body weight of 150–250 grams. The first group was normal to control with sufficient food, the second group was normal to control with poor nutrition, the third group was a negative control with insufficient food, the fourth group was positive control one was given Glibenclamide, the fifth group as positive control two was given Glibenclamide + Vitamin B6, sixth group was the treatment group with a dose of 50 mg, seventh group was the treatment group with a dose of 100 mg, and the last group was the treatment group with a dose of 150 mg.

After the rats were neurotoxic, which was marked by a significant decrease in the response to thermal stimulation, the rats were treated orally with Moringa leaf extract at doses of 50,100 and 150 mg/kg bb. After 7 days of given the extracted treatment, observations were obtained and the results were a decrease in blood sugar levels in rats. The speed of response to thermal stimulation also increases. Observations continued on days 14th and 21st after the extract treatment. The results of the observations were processed statistically and the results of the moringa leaf extract with a dose of 150 mg/kg bb showed there was no significant difference to the positive control of glibenclamide, but there was a significant difference in the positive control of glibenclamide + vitamin B6. Moringa leaf extract with a dose of 150 mg/kg bb showed the same activity as glibenclamide, but it was not better than glibenclamide + vitamin B6.

RESULT

Measurement of blood sugar levels in the optimization test when neurotoxicity occurred, rats were induced by STZ-Na intra peritoneally. Before being induced, the rat's blood sugar levels were measured, then on the third day the measurements were taken again and compared with the results of the previous observations. Measuring sugar levels regularly every seven days until the rats are declared to have diabetic neuropathy by paying attention to other parameters, namely observing the response to thermal stimulation.

Table 1. The changes in average blood sugar levels on day 1 to day 42 optimization of neuropathy induction and percentage of decrease in blood sugar levels

GROUP	Day to -								Δ H42-H1	% Δ H42-H1
	1	3	7	14	21	28	35	42		
I	20.6 ±1.85	20.6 ±1.85	20.8 ±1.94	20.8 ±1.6	20.6 ±1.5	20.6 ±1.5	20.4 ±1.36	20[a] ±1.41	11.00	15.67
II	19.9 ±1.43	20.2 ±1.17	19.6 ±1.36	19.6 ±1.02	18.8 ±0.75	18.2 ±0.75	18.2 ±0.75	18.2[a] ±0.75	-1.20	-1.56
III	20.2 ±0.75	20 ±0.63	19.6 ±0.8	19.6 ±0.8	19.4 ±1.02	19 ±1.1	18 ±1.41	16.6[b] ±1.02	117.80	152.20
IV	20.2 ±1.33	19.8 ±0.98	19.4 ±1.02	19.4 ±1.02	19.2 ±1.72	18.6 ±1.2	16.8 ±0.75	16[b] ±0.89	124.20	152.96

Information: Group I: Normal rat group, Group II: Group of malnourished rats, Group III: Normal rat group + Streptozotocin – Nicotinamide, Group IV: Malnourished rat group + Streptozotocin – Nicotinamide; *One Way Anova reading: a. significantly different from the normal+STZ-NA group and the malnutrition+STZ-NA group, b. significantly different from the normal, malnourished group.

The first tail flick pain response test was carried out before the rats were induced by STZ-Na. Retention time was recorded as the basis for comparison of the decrease in retention time due to neurotoxic conditions due to hyperglycemia. After the mice were declared to have hyperglycemia, the measurement of the tail flick pain response was always carried out every 7 days. This was done to find out on what days the rats experienced a decrease in their ability to respond to heat stimuli and were even unable to respond to heat stimuli due to neurotoxicity, as shown in Table 2.

Table 2. The changes retention time of tail flick on day 1 to day 42 optimizing induction neuropathy and percentage of decrease retention time

GROUP	Day to-								Δ H42-H1	% Δ H42-H1
	1	3	7	14	21	28	35	42		
I	8.6 ±1.02	7.8 ±0.75	8 ±0.63	8.6 ±1.02	8.6 ±0.49	8.8 ±0.75	8 ±0.89	8.2 ±0.75	-0.40	-4.65
II	8.6 ±0.8	7.2 ±0.75	8 ±0.89	7.6 ±1.02	8 ±0.89	9.2 ±0.75	9.4 ±0.49	9.8 ±0.75	1.20	13.95
III	8.2 ±0.75	6.8 ±0.75	7.4 ±1.02	6 ±0.63	10.8 ±1.33	12.2 ±1.17	14.8 ±1.94	14.8 ±2.04	6.60	80.49
IV	7 ±1.1	7 ±1.1	6.6 ±1.02	5.4 ±1.02	11.2 ±1.47	13.6 ±1.5	16.6 ±0.49	19 ±0.89	12.00	171.43

Information: Group I : Normal rat group, Group II : Group of malnourished rats, Group III: Normal rat group+streptozotocin-nicotinamide, Group IV: malnourished rat group+ Streptozotosin – Nicotinamide.

The first hot plate test was carried out when the rats had not been induced by STZ-Na as T1 which would be used as a comparison with the retention time after experiencing hyperglycemia. The hot plate test was continued when the rats had diabetes, namely on the third day and thereafter, they were repeated regularly every seven days. Measurements were stopped after the mice gave a significant decrease in pain response as a sign that neurotoxicity had occurred. According to Table 3, the results of the observations were processed using statistics to determine the significance of the decrease in retention time with the hot plate test.

Table 3. The changes of retention time average hot plate on day 1 to day 42 optimizing induction neuropathy and percentage of decrease retention time

Group	Day to-								Δ H42-H1	% Δ H42-H1
	1	3	7	14	21	28	35	42		
I	3.8 ±1.17	2.8 ±0.75	3 ±1.10	3.6 ±0.80	3 ±0.63	3 ±1.10	3.4 ±1.02	3[b] ±1.10	-0.80	-21.05
II	4 ±0.89	3 ±0.89	3.4 ±1.02	3.8 ±1.17	5 ±0.63	5.4 ±0.49	5.6 ±0.49	5.4[a] ±0.49	1.40	35.00
III	3.4 ±1.02	3.4 ±1.02	4 ±0.89	2.8 ±0.75	5.6 ±1.02	9.6 ±0.80	13.6 ±1.36	14.4[a] ±1.36	11.00	323.53
IV	3.4 ±1.02	3.2 ±0.75	3.6 ±0.80	3.2 ±1.17	9.4 ±0.49	12 ±0.89	13.6 ±1.02	16.2[a] ±0.75	12.80	376.47

Information: Group I: Normal rat group, Group II: Group of malnourished rats, Group III: Normal rat group + Streptozotosin – Nikotinamid, Group IV: malnourished rat group + Streptozotosin – Nikotinamid. *Reading One Way Anova: a. significantly different from the normal.

Measurement of rat blood sugar levels in the Moringa leaf ethanol extract test was conducted to determine whether Moringa leaf ethanol extract can lower blood sugar levels, as well as a parameter for the improvement of neuropathic nerve cells due to diabetes. Before the STZ-Na induction, the rat's blood sugar level was measured as T1 which will then be used as a comparison to whether the STZ-Na induction was successful. On the third day, sugar levels were measured again as T1 and then regularly measured every 7 days to see if after being given extract treatment, there would be a decrease in sugar levels as an indicator of neuroprotector activity of Moringa leaf ethanol extract.

Table 1. The changes in average blood sugar levels on the 1st day to the 49th day of the Moringa leaf ethanol extract test and the percentage decrease in blood sugar levels

GROUP	Day to-									Δ H49-H1	% Δ H49-H1
	1	3	7	14	21	28	35	42	49		
I	70.2 ±7.52	69.6 ±7.34	81.2 ±11.3	81 ±10.06	80 ±7.82	81.4 ±7.81	79.6 ±7.76	81.2 ±6.62	81[a] ±6.07	10.80	15.38

GROUP	Day to-									Δ H49-H1	% Δ H49-H1
	1	3	7	14	21	28	35	42	49		
II	77 ±10.33	76 ±10.53	78 ±8.12	77.8 ±7.78	75.6 ±6.62	76.4 ±4.5	76 ±4.56	75.8 ±4.75	75.6[a] ±4.27	-1.40	-1.82
III	81.2 ±6.05	216.2 ±4.26	208 ±11.7	212.8 ±9.58	214.4 ±9.48	209.2 ±8.23	208 ±7.56	205.4 ±7.14	203.6[b] ±8.01	122.40	150.74
IV	84.6 ±7.81	212.4 ±7.42	213.4 ±5.78	212.8 ±6.49	210.8 ±5.53	210.2 ±5.71	163 ±5.1	149.8 ±8.7	155.4[b] ±1.85	70.80	83.69
V	81.6 ±8.55	217.2 ±4.92	217 ±4.34	213.8 ±5.19	210.2 ±5.04	206.6 ±5.35	163.6 ±3.26	155.8 ±4.53	156.2[b] ±2.32	74.60	91.42
VI	84.2 ±11.09	215.4 ±3.61	215.4 ±3.67	215.4 ±3.5	208.4 ±4.63	208 ±6.96	189.6 ±4.5	183.2 ±5.56	178.8[b] ±6.46	94.60	112.35
VII	75.4 ±9.65	216.2 ±3.82	218 ±3.58	216.4 ±3.44	217.8 ±2.79	215.6 ±2.33	169.8 ±1.72	158.4 ±3.07	155.6[b] ±4.5	80.20	106.37
VIII	76.8 ±9.79	215.2 ±5.78	216 ±5.1	214.6 ±3.44	214 ±6.32	211.8 ±6.62	167.4 ±1.5	159.4 ±3.38	153.4[b] ±4.13	76.60	99.74

Information: Group I: Normal rat group, Group II: Group of malnourished rats, Group III: malnourished rat group + Streptozotosin – Nicotinamide, Group IV: Glilibenklamid rat group + Streptozotosin – Nicotinamide, Group V: Glibenklamid+Vit. B_6 rat group+ Streptozotosin – Nicotinamide, Group VI: Ekstrak Etanol moringa leaves 50 rat group + Streptozotosin – Nicotinamide, Group VII: Ekstrak Etanol moringa leaves 100 rat group+ Streptozotosin – Nicotinamide, Group VIII: Ekstrak Etanol moringa leaves 150 rat group+ Streptozotosin – Nikotinamid; *Pembacaan One Way Anova: a. significantly different toward the negative group, glibenclamid, glibenclamid+vitB6, extract 50mg, extract 100 mg, extract 150 mg, b. significantly different toward normal group, normal malnutrition.

The results of measuring blood sugar levels showed that rats in groups 7 and 8 experienced a decrease in blood sugar levels with almost the same percentage as groups 4 and 5, which can be seen in Table 4. This showed that group 7 was given 100 mg of Moringa leaf ethanol extract and group 8 was given Moringa leaf extract, 150 mg had a decrease that was almost close to group 4, namely the positive control of glibenclamide and the positive group of glibenclamide + vitamin B6. Meanwhile, group 6 which was given 50 mg Moringa leaf ethanol extract, was able to reduce blood sugar levels but was not better than the glibenclamide positive control group or the glibenclamide + vitamin B6 positive control group.

The first measurement of tail flick retention time was carried out before the rats were induced with STZ-Na as T1, then performed regularly every 7 days until the rats were neurotoxic. After the rats were declared to be neurotoxic, the rats were given ethanol extract of Moringa leaves and measured again every 7 days to see if there was an increase in the speed of retention time in the tail flick test. The existence of a neuroprotector effect on the ethanol extract of Moringa leaves can be seen if after administration of the extract, there is an increase in retention time. The faster the rats flicked their tails, indicating the repair of nerve cells damaged by hyperglycemia.

Table 5. The changes of time average retention tail flick on day 1 to day 49 extract test and percentage of decrease retention time

GROUP	Day to -									Δ H49-H1	% Δ H49-H1
	1	3	7	14	21	28	35	42	49		
I	8.6 ±1.02	7.8 ±0.75	8 ±0.63	8.6 ±1.02	8.6 ±0.49	8.8 ±0.75	8 ±0.89	8.2 ±0.75	8.2 ±0.9	-0.60	-6.98
II	8.6 ±0.8	7.2 ±0.75	8 ±0.89	7.6 ±1.02	8 ±0.89	9.2 ±0.75	9.4 ±0.49	9.8 ±0.75	9 ±0.63	0.40	4.65
III	7 ±1.1	7 ±1.1	6.6 ±1.02	5.4 ±1.02	11.2 ±1.47	13.6 ±1.5	16.6 ±0.49	19 ±0.89	18 ±0.89	11.00	157.14
IV	5.4 ±0.49	6.2 ±0.4	6.4 ±0.49	5.4 ±0.49	10 ±0.89	14.2 ±0.75	13.8 ±0.75	9.8 ±0.75	9 ±0.63	3.60	66.67
V	5.4 ±1.02	5.8 ±0.75	6.6 ±1.02	6.6 ±0.49	9.4 ±0.49	14.2 ±0.75	12.6 ±0.49	8.2 ±0.4	7.2 ±0.75	1.80	33.33
VI	5 ±0.63	5 ±0.63	6 ±0.63	5.4 ±0.49	9 ±0.63	15 ±0.63	14.8 ±0.75	13 ±1.1	9.6 ±0.49	4.60	92.00
VII	5.4 ±0.8	5.8 ±0.75	6.2 ±0.75	5 ±0.71	8.8 ±0.75	13.8 ±0.75	13.2 ±0.4	10 ±0.63	8.8 ±0.4	3.40	62.96
VII	5 ±0.63	5.6 ±0.8	5.8 ±0.4	5.2 ±0.4	9 ±1.1	14.6 ±0.49	13.6 ±0.49	9 ±0.63	6.6 ±0.49	1.60	32.00

Reading One Way Anova: a. significantly not different toward the normal group.

The hot plate test on the extract treatment was carried out with the aim of seeing whether the ethanol extract of Moringa leaves had a neuroprotective effect on rats that had experienced neurotoxicity. The first hot plate test was performed before the rats were induced by STZ-Na, the retention time was recorded as T1. Furthermore, the hot plate test was carried out on the third day after STZ-Na induction when the rats had experienced hyperglycemia, then the test was carried out regularly until the rats were neurotoxic. After experiencing neurotoxicity, the rats were given ethanol extract of Moringa leaves and then the hot plate was tested again to determine whether there was an increase in retention time against hot plate thermal stimulation, according to Table 6.

Table 6. The changes of time average retention hot plate on day 1 to day 49 extract treatment and percentage of decrease retention time

GROUP	Day to-									Δ H49-H1	% Δ H49-H1
	1	3	7	14	21	28	35	42	49		
I	3.8 ±1.17	2.8 ±0.75	3 ±1.10	3.6 ±0.80	3 ±0.63	3 ±1.10	3.4 ±1.02	3 ±1.10	2.6[a] ±0.80	-1.20	-31.58
II	4 ±0.89	3 ±0.89	3.4 ±1.02	3.8 ±1.17	5 ±0.63	5.4 ±0.49	5.6 ±0.49	5.4 ±0.49	4.8[ab] ±0.75	0.80	20.00
III	3.4 ±1.02	3.2 ±0.75	3.6 ±0.80	3.2 ±1.17	9.4 ±0.49	12 ±0.89	13.6 ±1.02	16.2 ±0.75	15.6 [b] ±1.02	12.20	358.82
IV	2.6 ±0.80	2.6 ±0.80	4 ±0.89	3.4 ±0.49	9 ±0.63	11.8 ±0.75	11.4 ±0.49	9.6 ±0.49	8.8[ab] ±0.40	6.20	238.46

GROUP	Day to-									Δ H49-H1	% Δ H49-H1
	1	3	7	14	21	28	35	42	49		
V	2.6 ±0.49	2.6 ±0.80	4 ±0.63	3.4 ±0.49	8.6 ±0.49	13.4 ±0.49	12 ±0.63	7.8 ±1.17	4.8[ab] ±0.75	2.20	84.62
VI	2.6 ±0.49	3.2 ±0.75	4.6 ±0.49	3.8 ±0.40	9 ±1.10	13.2 ±0.75	12.6 ±0.49	10.8 ±0.40	10[ab] ±0.63	7.40	284.62
VII	2.8 ±0.75	4 ±0.63	4.4 ±0.49	4.6 ±0.49	9.6 ±0.80	12.8 ±0.75	11.4 ±0.49	10.2 ±1.17	7.8[ab] ±0.75	5.00	178.57
VII	2.6 ±0.49	4.4 ±0.49	4.4 ±0.49	5.2 ±5.20	9.6 ±0.80	13 ±0.63	11.2 ±0.75	9 ±0.63	5.4[ab] ±0.49	2.80	107.69

*Reading One Way Anova: a. significantly different toward normal group, b. significantly different toward malnutrition group STZ-NA.

The results of the hot plate test on the rat group with the extract treatment showed that the rat group VI experienced an increase in retention time, but it was not better than groups IV and V. This means that at a dose of 50 mg Moringa leaf ethanol extract can increase the pain response in the soles of the rats as a sign of improvement in neuropathic nerve cells, but not better than glibenclamide or glibenclamide + Vitamin B6. Group VII rats also experienced an increase in pain stimulus response with a better percentage when compared to group IV rats that were given glibenclamide alone. However, it was not better than group V rats given glibenclamide + Vitamin B6. The results of the hot plate test on group VIII rats showed better results than those on group IV and group V rats. That is, the group of rats treated with 150 mg extract was able to work better than glibenclamide but not better than glibenclamide + vitamin B6.

DISCUSSION

Oxidative stress is a biochemical trigger that leads to nerve dysfunction and reduced blood flow in diabetic rats, causing damage to nerve cells. In addition, nerves experience a reduction in glutathione and glutathione peroxidase activity. Neuropathy is a progressive process, where there is degeneration of nerve fibers causing symptoms such as pain or numbness. The nerves that are usually affected are those in the legs or arms. Neuropathy is caused by nerve structure damage and dysfunction due to increased polyol pathways, decreased myoinositol formation, and decreased Na/K ATPase, resulting in segmental demyelination or axonal atrophy.

The ethanol extract of Moringa Oleifera, which has antioxidant content, has been associated with an improvement in nerve function in hyperglycemic conditions. The extract contains flavonoids, tannins, anthraquinones, triterpenoids, saponins, and reduced sugars. Flavonoids in Moringa Oleifera can function as insulin secretagogues or insulin mimetics, minimizing diabetes complications. Moreover, the flavonoid compounds in Moringa Oleifera are beneficial for lowering blood sugar levels and have excellent antioxidant abilities, thus potentially restoring nerve function damaged by high blood sugar levels.

CONCLUSION

The results of the research that has been carried out on the ethanol extract of Moringa leaves, it can be concluded that the ethanolic extract of Moringa leaves with doses of 50, 100, and 150 has antihyperglycemic activity. There was a difference in the timing of neurotoxicity in

normal and malnourished DM rats. In normal-feeding rats, the neurotoxicity period was 35 days, while in the nutritionally deficient group of rats, the neurotoxicity occurred on day 28 after STZ-Na induction. Moringa leaf ethanol extract at a dose of 50 mg did not provide better activity than glibenclamide or glibenclamide + Vitamin B6. Moringa leaf ethanol extract doses of 100 mg and 150 mg gave better activity than the Glibenclamide group, but not better than the glibenclamide + Vitamin B6 group. The most potent dose of Moringa leaf ethanol extract to exert a neuroprotective effect on STZ-Na-induced type 2 DM was at a dose of 150 mg.

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