Omni Health Journal
e-ISSN: 3047 - 6216
Volume 1 Issue 3: page 76-84, October 2024

Original Research

Effect of Ethanol Extract of Nutmeg Leaves (Myristica Fragrans) on Hispathotic Features of Diabetic Rats (Ratus Norvegicus)

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Article Info

Received: 24-07-2024
Revised: 27-08-2024
Accepted: 08-09-2024

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Abstract

Background: Nutmeg leaves (Myristica fragrans) are used by the community to treat some diseases such as pain. Chemicals contained in nutmeg leaves such as saponins, polyphenols, flavanoids, and essential oils. To prove the activity of ethanol extract of nutmeg leaves (myristica fragrans) against the aorta of diabetic rats (rattus norvegicus).
Objective: To prove the activity of nutmeg leaf ethanol extract (myristica fragrans) against the aorta of diabetic rats (rattus norvegicus), and prove the activity of nutmeg leaf ethanol extract (myristica fragrans) on hispathological features of foam cells and aortic diameter of diabetic rats (rattus norvegicus).
Methods: This research is a type of research that is laboratory experiments (laboratory experiments) in vivo using experimental animals of white rats (rattus norvegicus) male wistar strains.
Results: Nutmeg leaves use 96% ethanol solvent. Maceration results of 300 g were obtained, concentrated with a rotary evaporator device obtained a thick extract of 42.673 g with a soaking % of 14.23%. Histological images were obtained of thinner and wider diameter lumen and foam cells in the tunica intima and aortic media of mice.
Conclusion: From the results of research on the effect of ethanol extract of nutmeg leaves (myristica fragrans) on the hispathotic picture of aortic rats (rattus norvegicus) diabetes. To prove the activity of ethanol extract of nutmeg leaves (myristica fragrans) on hispathological features of foam cells and aortic diameter of diabetic rats (rattus norvegicus).
Keywords: nutmeg leaves (Myristica Fragrans); Rat Aorta (Rattus norvegicus); Diabetic

Introduction

Diabetes Mellitus (DM) is a group of metabolic diseases with hyperglycemia characteristics that occur due to abnormalities in insulin secretion, insulin action, or both. The classification of DM generally consists of DM type 1 or Insulin Dependent Diabetes Mellitus (IDDM) and DM type 2 or Non Insulin Dependent Diabetes Mellitus (NIDDM). Type 2 diabetes occurs because cells β pancreas produce small amounts of insulin or experience insulin resistance. The number of people with type 1 DM is 5-10% and type 2 DM is 90-95% of DM sufferers worldwide (ADA, 2020).

Diabetes Mellitus (DM) as a global problem continues to increase in prevalence from year to year both in the world and in Indonesia. Based on International Diabetes Federation (IDF) data, the global prevalence of DM in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) in 2030 and 10.9% (700 million) in 2045 (IDF, 2019). In 2015, Indonesia ranked 7th as the country with the most DM sufferers in the world, and is expected to rise to 6th place in 2040 (Perkeni, 2019).

Based on data from the International Diabetes Federation (IDF) in 2017, 425 million people with diabetes mellitus are expected to increase by around 48% with 629 million people with diabetes mellitus. It is estimated that the prevalence of 151 million people with diabetes mellitus in Southeast Asia is estimated to increase from 82 million people with diabetes mellitus in 2017 (IDF 2017).

Symptoms of coronary heart disease in people with diabetes mellitus can be clearly seen but can also not appear until finally the person experiences sudden death. Usually the manifestations that can appear in the form of symptoms of angina pectoris are chest pain, can be in the form of heaviness, sliced feeling, such as squeezing, feeling crushed heavily, on the left or middle chest which can radiate to the neck, shoulders, back or left arm. Patients can also complain of shortness of breath, fatigue, tightness during activities or dyspeptic syndrome such as heartburn, nausea or vomiting. This complaint can arise at rest or during activities (Lena 2013).

Atherochloresis (ATH) is a blood vessel wall disorder that can progress to plaque that greatly disrupts blood vessel flow if large enough. The most affected arteries are the coronary, aorta, and cerebral arteries. Exposure to free radicals in endothelial cells of arterial walls causes LDL oxidation. LDL oxidation

can be captured by macrophages through scavenger receptors, when exposed to LDL oxidized macrophages into foam cells. The accumulation of foam cells or known as fatty stereacs which is a pull of fat that is macroscopically invisible but microscopically visible as foam cells occurs in the subendothelium of the vessel and this is the earliest evidence of atherochloresis plaque growth. Atherochloresis will affect the diameter and picture of aortic hispathology damage (Gurujaj HB, 2013).

Based on the average data of Indonesian nutmeg production in 2012-2016, nutmeg production centers in Indonesia are located in 5 (five) provinces, namely Aceh, North Maluku, North Sulawesi, Maluku and West Papua. The five provinces contributed a cumulative contribution of 86.71%. Aceh ranks first with a contribution of 25.46% per year. The second rank is occupied by North Maluku with a contribution of 19.89% per year, followed by North Sulawesi, Maluku and West Papua with a contribution of 14.79%, 14.65% and 11.93% respectively while the contribution of production from other provinces is 13.29% (BPS, 2017).

Nutmeg is a very important antioxidant for boosting immunity during the fall months and flu season, as well as preventing other chronic diseases. Antioxidants are compounds that protect cells from free radicals, which are molecules linked to heart disease, cancer, and other diseases. Nutmeg may provide anti-inflammatory benefits for people living with conditions such as diabetes, heart disease, and arthritis (Phytochemistry reviews, 2016).

Nutmeg leaves have a pharmacological effect treating diarrhea, muntaber, scabies. Some chemicals contained in nutmeg leaves include saponins, polyphenols, flavanoids, and essential oils (Hariana 2013). Nutmeg leaves contain chemicals including saponins, tannins, flavanoids, steroids / triterpenoids, polyphenols and essential oils (hariana 2013). Agoes (2010) said nutmeg flesh is rich in calcium, phosphorus, vitamin C, vitamin A and a little iron.

Based on the above background, the author is interested in conducting a study entitled "The effect of ethanol extract of nutmeg leaves (myristica fragrans) on the image of aortic hispathology of diabetic rats (rattus norvegicus)".

Methods

Study Design

This research is a type of research that is laboratory experiments (laboratory experiments) in vivo using experimental animals of white rats (rattus norvegicus) male wistar strains. The study used a post-test control group design research design.

Samples/Participants

25 wistar rats that meet the inclusion criteria such as, male wistar ikus, age 2-3 months, bb 150-200 grams, healthy. The sample size is calculated using the Federer formula where a sample of 25 is obtained

Instruments

The ingredients in this study were nutmeg leaf extract (myristica fragrans), glibenclamide 5 mg, alloxane 18-24 mg as much as 0.2-0.3 ml, aquades. As for this Research Instrument, namely, rat maintenance equipment (rat cage, rat cage cover from woven wire, water bottles, scales, rat feeding places, gloves, kendang racks), extract making tools (scales, ovens, erlemeyers, glass funnels, filter paper, evaporator flasks, water pumps , water bath, vacuum pump, bottle), hispathology examination equipment (glas object, dec glas, rat surgical tool, oaraffin board, tweezers, scissors, needle bounce, streoform, label paper, hermetically sealed plastic container containing enter, 5cc dispo, vacontener).

Interventions

Adaptation stage

All mice were adapted to the environmental conditions of the study for 1 week by being fed feed without heating and drinking water ad libitum. The rats were placed in a clean, quiet room with good ventilation, with regulated temperature and humidity and free of stimulating odors. The cage used is 900 cm for 4-5 mice. The cage is cleaned at least once a week with cleaning drugs or hot water. The bedding of the cage is used dust-free sawdust, changed at least once a week or if ammonia smells. Clean drinking water is provided in plastic bottles so that rats can drink at any time. Drink bottles are cleaned once a week.

Induction stage

Rats were randomly divided into 5 groups, each group consisting of 5 mice. All groups were induced alloxane monohydrate at a dose of 120mg/200kgBB intraperitoneally (I.P) in male white rats (rattus norvegicus), except group I (positive control). After measuring the blood sugar levels of the rats, so that blood sugar levels rose then given oral treatment. Group I (positive control), the group that was not given treatment. Group II (comparison control), the group given glibenclamide 0.09 mg/200kgBB. Group III group given nutmeg leaf extract (myristica fragrans) dose I (100 mg / kg BB). Group IV group given nutmeg leaf extract (myristica fragrans) dose II (200 mg / kg BB). Group V group given nutmeg leaf extract (myristica fragrans) dose III (300 mg / kg BB).

Stages of surgery
After the animal is well anesthetized (fainting), the animal is placed on the oaraffin papa and the four legs of the experimental animal are fixed against the oaraffin board using a bounce needle. Using a scalpel, surgery is performed on the abdomen to take the aortic organ.

Data Collection
Selected male wistar rats aged 2-3 months, body weight 150-200 grams as much as 25 heads. Kept in cages covered with nets and husk-lined with chaff. Rats were adapted for 7 days and fed and watered After adaptation was randomly selected, grouped into 5, each group was given 0.2-0.3 ml alloxane and evaluated selma 2-3 days. Group I (positive control): group that was not treated. Group II (comparison control): group given glibenclamide 0.09 mg/200kgBB. Group III group given nutmeg leaf extract (myristica fragrans) dose I (100 mg / kg BB). Group IV group given nutmeg leaf extract (myristica fragrans) dose II (200 mg / kg BB). Group V group given nutmeg leaf extract (myristica fragrans) dose III (300 mg / kg BB).

Data Analysis
The data obtained from the experimental results are displayed in tabular form, then analyzed using the statistical method One Way ANOVA with LSD. Data processing using SPSS type 24.0 for windows with a confidence level of 95%.

Ethical Considerations
Research ethics was obtained from the ethical Institute of the College of Health Sciences Maluku Husada

Results
Extraction
The result of making simplicia from 5 kg of fresh nutmeg leaves was obtained from 2 kg of dried simplisa. The result of maceration is 300 gr of nutmeg leaf simplicia powder using 96% ethanol solvent. Concentrated with a rotary evaporator, a thick extract of 42.673 g was obtained with a soaking of 14.23%.

Table 1 Results of Nutmeg Leaf Powder

Weight Powder	Characteristic			
	Shape	Color	Construction	% Rendamen
300 gram	The State	Black	Khas	
42,673 gram	Kental	Dark green	Khas	
300 gram				14,23%

Source: Primary Data 2021

Screening Phytokimia

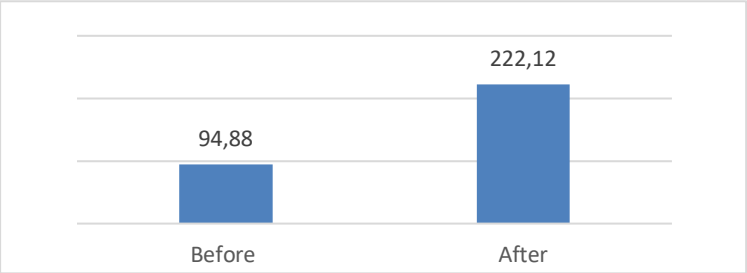
Tablet 2 Harvest Screening

Examination	Treatment	Hasil	Information
Alkaloid	Nutmeg leaf extract + dragendroff reagent + H2SO4	+	Formed yellow color
Flavonoid	Nutmeg leaf extract + HCL +magnesium	+	Formed yellow color
Terpenoid	Nutmeg leaf extract + liebermen-bouchardat reagent	+	Formed red color
Steroida	Nutmeg leaf extract + liebermen-bouchardat reagent	+	Formed red color
Fhenolik	Ekstrak daun pala + aquades + FeCL3	+	Formed blue color

Saponin / tanin	Nutmeg leaf extract HCL	+	+	Formed foam

Source: Primary Data 2021

Figure 1 Blood sugar levels before and after alloxane



The average blood sugar level before treatment was 94.88 mg/Dl and the average blood sugar level after treatment was 222.12 mg/dL. The average percent increase in blood sugar levels before and after the administration of allocan was 42%.

Sel Foam Aortic Rat Diabetes

Preparations were made with Hematosilin-Eosin staining to determine the number of foam cells and then slide observations were carried out using a light microscope with a 1000x ratio. Foam cells are seen by the onset of space pressure in the subintima and tunica of the aortic media. Calculations were made on ten different fields of view and then the average number was taken. The following is a table and graph of the average number of aortic foam cells in each treatment group.

Table 3 Average Number of Foam Cells

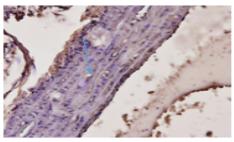
Group name	Number of foam cells (average ± sd)
Control negative	4,00 ± 0,406
Positive control	5,20 ± 0,406
T1 Treatment	3,00 ± 0,889
T2 Treatment	4,80 ± 0,600
T3 Treatment	1,60 ± 0,240

Source: Primary Data 2021

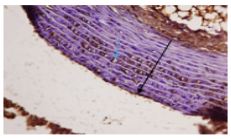
Figure 2 Average Foam Cell Count

According to the results of the diagram above, the negative group and the T3 treatment have the smallest average number of foam cells so that it can distinguish the normal number of mouse foam cells from the other four groups. The positive control group that was given glibenclamide and not treated in the form of nutmeg leaf extract had a larger average number of foam cells compared to K(-), PT1, PT2 and PT3. The T1 treatment group, the T2 treatment, and the T3 treatment group experienced a decrease in the average number of foam cells when compared to the average result of the number of foam cells in the positive control group. The T3 treatment group was the group that experienced the most average decrease in the number of foam cells.

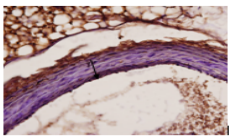
Figure 3 The results of aortic histopathology observations in the treatment group are presented in the figure below



Gambar A terbentuk sel busa



Gambar B diameter lebih tebal dan terlihat sel busa



Gambar C diameter lebih tipis

Based on the results of histopathological observations of the rat aorta, it can be seen that in the rats of figure A, there are foam cells formed. Similar things can be seen in the histopathological results of image B and image C of diabetic rats. In the diabetic group, the presence of foam cells and diameter was observed.

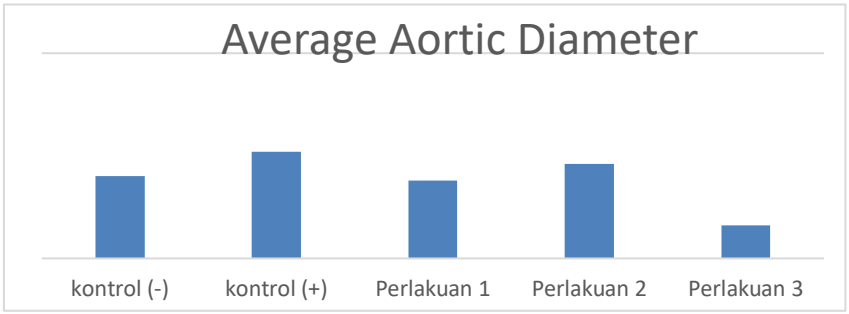
Diameter of the aorta of diabetic rats

The measurement of aortic thickness was carried out using aortic preparations that had been stained with hematoxilin-eosin. Slide observation was carried out using a 1000x microscope. The cross-sectional of the aorta was measured with a micrometer at ten different points on the transverse section of the lumen of the aorta and then averaged the results

Table 4 Average Aortic Diameter

Test group	Average	Sd	Min.	Mak.
Control (-)	4,00	2,73	1	8
Control (+)	5,20	1,97	3	8
T1 Behaviour (100 grBB/day)	3,80	2,38	1	7
T2 Behaviour (200 grBB/day)	4,60	2,40	2	8
T3 Behaviour (300 grBB/day)	1,60	1,51	0	4

Figure 4 Average Aortic Diameter



From this research, the results of the aortic diameter in the (+) group experienced a higher diameter thickness when compared to other groups, the (-) group had a thicker aortic diameter when compared to PT1 and PT3 while for PT2 the aortic thickness was not much different from the control (-), PT1 the aortic diameter was thicker when compared to PT3, PT2 the aortic diameter was thicker when compared to PT1 and PT3, PT3 aortic diameter thickness is smaller when compared to K(+), K(-), PT1, PT2.

Discussion

Extraction

In this study the sample used was nutmeg leaves (myristica fragrans) taken from trees in Tulehu village, simplisia was taken by picking. Simplisia is taken in the morning because the leaves are collected during flowering and before ripening, nutmeg leaves reach the highest levels of alkaloids on the shoots of the plant when they begin flowering. Photosynthetic plants are taken leaves during the perfect photosynthesis reaction, which is 09.00-12.00 and after that simplisia is cleaned under running water to separate dirt and foreign materials such as soil, gravel, grass. After that simplisia is dried, simplisia drying is done by aerating because the dryer has a real effect on the moisture content of the material, this is in line with the work done by Winangsih (2015) where the drying method has a significant effect on the dry weight of simplisia. Drying is done to remove water from the leaves and obtain simplisia that is not easily damaged. This reduction in water content is carried out to prevent the growth of mold in simplisia and reduce enzymatic reactions that can cause damage to simplisia (Maulidiyah, 2018).

Simplisia dried nutmeg leaves are then pureed using a blender then stored in a plastic bag and placed in a dry, not damp place and protected from direct sunlight, this is done to protect the simplisia from being damaged or changing its quality (Maulidiyah, 2018).

In this study, nutmeg leaves weighed as much as 300 grams, added 96% ethanol solvent as much as 2000 liters and then soaked for 3 days while occasionally stirring at room temperature and protected from light. Extraction is carried out with the aim of pulling out the active compounds contained in nutmeg leaves. The amount of active ingredient extract obtained is influenced by the soaking time of the extracted material and stirring (Yumas, 2017).

The results of nutmeg leaf powder obtained were 300 grams, then extracted and produced extracts as much as 42.673 grams, and the calculation result of % Marinade 14.23%.

This research was carried out by maceration method because the maceration method uses equipment that is very simple and relatively cheap and easy to obtain, the working technique is relatively simple and easy to do, the operational costs are relatively low, can be used to extract thermolabile compounds because maceration is done without heating and is very good for use in samples that have not known carарcterization of compounds (Sinta dewi, 2020) extraction is carried out using solvents. The solvents used in the maceration method are generally non-aqueous solvents or semi-polar or non-polar solvents. When the sample is immersed in a solvent, the filter liquid will penetrate the cell wall and enter the cell full of active substance and because there is a meeting between the active substance and the filter, the dissolution process occurs, then the concentrated solution will be pushed out. This event will occur repeatedly so that a balanced concentration is achieved between the solution outside and inside the cell (Sinta dewi, 2020).

The solvent used to extract nutmeg leaves is ethanol 96% is the polar solvent most widely used to extract natural materials and known as a universal solvent, ethanol can extract more active compounds compared to other types of solvents (Nurjannati, 2018).

The maceration results are evaporated using a rotary evaporator at a temperature of 30 ° C with low pressure, evaporation is carried out until there is no more dripping solvent and a thick extract is obtained, the evaporation process is carried out with the aim of removing solvents from the extraction results until pure acstrak can be obtained (Nurjannati, 2018).

Phytochemical Identification Test of Nutmeg Leaves

Phytochemical testing is carried out to determine the chemical components contained in nutmeg leaves (myristica frangrans). First, an alkaloid test is carried out, namely by taking as much as 0.5 grams of extract inserted into a test tube then the sample is added with 3 drops of dragendroff reagent and then added 11 drops of H2SO4, the extract if a red precipitate is formed on the drangendroff perectation, it is positive for the presence of alkaloids. From the results of the treatment of the test solution shows positive results because a yellow color is formed in the test solution.

Second, flavonoid extract test samples as much as 0.5 grams were taken and inserted into test tubes. Then the sample was added 2 mg magnesium powder and given 3 drops of concentrated HCL. The sample was shaken and observed changes that occurred in the formation of yellow or pale yellow indicating the presence of flavonoid content. The treatment showed positive results because it formed a pale yellow color.

Third, a terpenoid test was carried out, a sample extract of 0.5 grams was taken and inserted into a test tube. Then added 3 drops of liebermen reagent – bouchardat, left for 15 minutes if red color appears indicating the presence of terpenoid content. The treatment showed positive results because it formed a red color.

Fourth, a steroida test was carried out as much as 1 gram of extract into a test tube, add Lieberman-buchard reagent. If a red color is formed, it indicates the presence of steroidal content.from the treatment shows positive results because it is formed red.

Fifth, a pehnolic test of 0.5 grams of sample extract was taken and put into a test tube, adding aquades and 1% FeCL3. Positive extract contains tannins if blue color is formed And treatment shows positive results because blue color is formed

Sixth, a tannin saponin test was put 0.5 grams of extract into a test tube, add aquades, then shaken for approximately 1 minute and let stand for 10 minutes, then add HCL 2N. If it causes foam or foam, it indicates the content of saponins.from the treatment shows positive results because foam is formed.

Making Diabetic Rats

In this study, the manufacture of diabetes in rats was done by inducing alloxane intraperitionally in mice. Aloxane administration is a quick way to produce diabetic conditions in experimental (hyperglycemic) rats in experimental animals. Induction is done by intraperitional injection, namely by injecting alloxane in the abdomen (stomach) of mice. The reason for intraperitional use is because it is more beneficial when compared to sonde because alloxane directly enters the animal's stomach (Maulidiyah, 2018) in this study the dose of alloxane used was 30 mg / rat 200 grams (Ayu rochmawati, 2018).

The reason for using aquades is because aquades are used as a negative control to compare glibenclamide and nutmeg leaf ethanol extract extract which affects changes in blood sugar rise in diabetic rats. The reason for the use of glibenclamide is because it is used as a drug to reduce blood sugar levels (diabetes).

Effect of Aloxane Induction on Blood Glucose Levels and Aortic Damage of Rats

Diabetes mellitus can be caused by many factors. These factors include genetic factors, infection by germs, nutritional factors, diabetogenic substances, and free radicals (oxidative stress). Aloxane compounds are one of the diabetogenic substances that are toxic, especially against pancreatic beta cells, and if given to experimental animals such as rats, it can cause experimental mice to become diabetic. The mechanism of alloxane toxicity begins with the entry of alloxane into pancreatic beta cells and the speed of uptake will determine the diabetogenic properties of alloxane. Damage to β cells occurs through several processes simultaneously, namely through oxidation of sulfidril groups and the formation of free radicals. The mechanism of action of alloxane produces damage to pancreatic β cells, especially attacking cellular compounds containing sulfidril groups, cysteine amino acids and proteins binding to SH groups (including enzymes containing SH groups). Aloxane reacts with two SH groups that bind to the side of the protein or amino acid to form disulfide bonds so as to inactivate the protein which results in impaired function of the protein (Szkuldelski, T. 2018). Induction of alloxane at a dose of 120 mg/kg bw intraperotoneally was able to increase blood glucose levels and damage to rat pancreatic β cells. Rats expressed hyperglycemia when blood glucose levels > 135 mg / dL.

The purpose of aortic histopathology observation is to find out in more detail about the effect of glibenclamide and nutmeg leaf extract (EDP) on the restoration of aortic function due to alloxane induction. This proves that alloxane administration can damage pancreatic endocrine cells, especially beta cells so that insulin secretion into blood vessels decreases (Nurdiana N.P. 2018). The aorta is a vessel carrying blood from the heart to the rest of the body. A decrease in the number of pancreatic beta cells indicates a disruption of insulin metabolism in the pancreas which causes a decrease in beta cell volume.

Conclusion

From the results of research on the effect of ethanol extract of nutmeg leaves (myristica fragrans) on the hispathotic picture of aortic rats (rattus norvegicus) diabetes. To prove the activity of ethanol extract of nutmeg leaves (myristica fragrans) on the hispathological picture of foam cells and aortic diameter of diabetic rats (rattus norvegicus).

Acknowledgment

We express our gratitude to all parties directly involved in this research.

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