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Long Le
Center of Science and Technology Development for Youth, 01 Pham Ngoc Thach Str, Ward Ben Nghe, District 01, Ho Chi Minh City, Vietnam

Ngoc-Anh Nguyen
Nguyen Hien High School, 03 Duong Dinh Nghe Str, Ward 8, District 11, Ho Chi Minh City, Vietnam

Ly-Thai-Uyen Thi
Nguyen Hien High School, 03 Duong Dinh Nghe Str, Ward 8, District 11, Ho Chi Minh City, Vietnam

Thi-Tan-Luong Nguyen
Nguyen Hien High School, 03 Duong Dinh Nghe Str, Ward 8, District 11, Ho Chi Minh City, Vietnam

Corresponding Author:
Long Le
Center of Science and Technology Development for Youth, 01 Pham Ngoc Thach Str, Ward Ben Nghe, District 01, Ho Chi Minh City, Vietnam

The effects of mangrove plant leaves (*Acanthus ilicifolius*) collected in can Gio district on white mice (*Mus musculus* var. albino) with diabetes

Long Le, Ngoc-Anh Nguyen, Ly-Thai-Uyen Thi and Thi-Tan-Luong Nguyen

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Abstract
This study was conducted to evaluate the hypoglycemic potential of mangrove plant leaves (*Acanthus ilicifolius*) fluid in white mice with diabetes mellitus with doses of mangrove leaves fluid of 100 mg/kg, 200 mg/kg, 300 mg/kg, 400 mg/kg corresponding to EX1, EX2, EX3, EX4, CT2. Diabetic mice had study results after 7 days. In EX1, EX2 and EX3 there was a decrease in blood glucose levels, after 7 days the results were 225.39 mg/dl respectively; 219.16 mg/dl and 201.55 mg/dl both had values greater than 200 mg/dl indicating that rats in these trials still had diabetes. In EX4, hypoglycemia with a dosage of 400 mg/kg mangrove leaves extract showed good therapeutic ability when blood glucose levels decreased to 178.36 mg/dl (<200 mg/dl). When studying, histological analysis of diabetic white mouse kidney on H&E staining specimens, kidney tissue cells, glomeruli had structural and functional changes. The above results show that mangrove plant leaves extract has the ability to lower blood glucose levels and protect kidney tissue and glomeruli from damage, limiting the level of kidney cancer caused by diabetes.

Keywords: Glomerulus, mice, kidney, diabete, mangrove

Introduction
Diabetes mellitus is an endocrine disease causing disturbance of carbohydrate metabolism found in most countries of the world and increasing rapidly (Skyler, 2004) [23]. Vietnam is considered as one of the Southeast Asian countries with a high prevalence rate of 3.8% of the population. Currently, in parallel with the treatment of diabetes with drugs of synthetic origin, the use of medicinal herbs of natural origin have been widely used in folklore because they are the available source of medicinal herbs in the world. Natural, cheap and easy to use (Akhtar and Ali, 1984) [1]. Therefore, the search and research on herbs capable of treating diabetes is being interested and developed by scientists around the world. The coastal mangrove plants have long been a rich and close source of natural medicine for people living in the coastal areas. Plants belonging to the Oraceae family live along the coasts and mangroves, and are abundant in Southeast Asia (Firdaus et al., 2013) [7]. Extracts from mangrove plant parts have been shown to contain antioxidant chemical compounds such as flavonoids, alkaloids, glycosides, lignans, saponins, triterpenoids, sterols, fatty acids and coumaric acid derivatives (Singh *et al*., 2009) [22]. In terms of anti-inflammatory ability, all of the above compounds present in mangrove leaves have anti-inflammatory properties. In particular, flavonoids found in this plant help promote fat cell differentiation and glucose absorption, which may be beneficial for diabetics. In addition, mangrove in folklore is used as many different remedies such as: clearing heat, detoxifying the body; has the ability to destroy inflammation, overcome the status of yellow urine; treatment of asthma, whooping cough; regulate and clear blood stasis; has the effect of supporting the treatment of jaundice, hepatitis caused by viruses; treat epilepsy, muscle spasms, treat bladder stones; inhibit the growth of cancer cells, prevent cancer; treat intestinal diseases; anti-inflammatory and other diseases. Scientists have suggested that the reason for choosing guinea pigs is that there are some fairly high genetic correlations with humans, and can study mice as representative of humans (Jonas, 1984; Yang *et al*., 2007) [10, 25].

On that basis, the topic "The effects of mangrove plant leaves (*Acanthus ilicifolius*) collected in Can Gio district on white mice (*Mus musculus* var. albino) with diabetes" was carried out to aim Evaluation of blood sugar lowering ability of mangrove leaves (*Acanthus ilicifolius*) collected in Can Gio.

Materials and Methods

Materials

White mice were raised for 3 days to stabilize the environment and ensure the raising conditions for the duration of the experiment. During the feeding period, mice were fed with hamster food, 3 times a day, both the nutrition and drinking water for the mice have to be ensured to meet the conditions of the experiment. Sea hollies were collected as whole trees (including, roots, stems, leaves) at 2 locations: Mangrove Plant Collection Garden and Dần Xây World Biosphere Reserve in Cần Giờ district, Hồ Chí Minh City. After an on-site identification image had been obtained and brought it back to the lab, the sea hollies were extracted for the experiment. Diabetes mellitus occurs over a long period of time, accumulating glucose for a long time, so in this study, the direct injection of glucose can with alloxan through the peritoneum cannot be used (Tsai *et al*., 2004) [24]. The study used regular glucose intake at a concentration of 5g/kg (Bich *et al*., 2022) [4]. Mice were kept in 6 cages, each with 7 mice, all mice were given sugar water with a glucose concentration of 5 g/kg, 3 times/day. Mice with blood glucose values averaged 238.17±0.12 mg/dl and all mice had glucose values greater than 210 mg/dl, this indicated that all mice had contracted diabetes. However, 2 mice had glucose values greater than 290 mg/dl and had necrotic ulcers caused by severe diabetes mellitus, so the study did not use these mice. After the diabetic results came back, all the mice were redistributed into 6 cages, each with 5 identical diabetic mice to see the possibility of mangrove leaves fluid. In addition, the topic analyzed the blood glucose and urine glucose values of 3 random mice that were not diabetic and were healthy from the start of the study. The mouse kidneys were also operated on as a basis for comparisons to other kidneys in the studies. To select the concentrations level, the study referenced the publication of (Flora *et al*., 2009; Ouarda *et al*., 2021; Sinaga *et al*., 2021) [8, 19, 21] to select the mangrove fluids concentrations of 100 mg/kg, 200 mg/kg, 300 mg/kg, 400 mg/kg. The study used Diabetna at a dose of 80mg/kg, which has a hypoglycemic effect to compare the effectiveness of mangrove leaves.

Negative control (CT1): untreated diabetic mice.

Test 1 (EX1): diabetic mice and mangrove fluids drink with a 100 mg/kg dose.

Test 2 (EX2): diabetic mice and mangrove fluids drink with a 200 mg/kg dose.

Test 3 (EX3): diabetic mice and mangrove fluids drink with a 300 mg/kg dose.

Test 4 (EX4): diabetic mice and mangrove fluids drink with a 400 mg/kg dose. Positive control (CT2): diabetic rats and oral ingestion of Diabetna 80 mg/kg.

Lab rats were tested again after 7 days.

Methods

On field mangrove tree sampling method in Can Gio district

Mangrove trees are studied in shape, size, and species identification prior to sample collection. At the Mangrove Plant Collection Garden and the World Biosphere Reserve, we searched for mangrove trees according to their pre-learned shapes and sizes. Samples of mangrove trees were collected in accordance with principles such as: Each sample must have a full range of parts, especially: branches, leaves, flowers and fruits. Then, perform identification imaging in the field where the samples were collected to ensure that once again that the samples collected were with the correct species of mangrove (*Acanthus ilicifolius*).

Method of collecting mangrove leaves

After performing the identification to select the right mangrove tree (*Acanthus ilicifolius*) collected in Can Gio mangrove forest, the plants were preserved so as not to wilt and sent to the laboratory to collect the leaves. The fluid extraction process is carried out as follows:

Step 1: Prepare a sample of fresh mangrove leaves, then the leaves were washed, after that, the thorns were removed through cutting.

Step 2: Blend the pure mangrove leaves with the ratio of 20g mangrove leaves: 200mL distilled water.

Step 3: Strain the mangrove leaves fluid to obtain the fluid used for the experiment.

Methods for evaluating hypoglycemia

Every morning when the mice were hungry, we measured their blood glucose levels with an Accu-chek active portable blood glucose meter to study the use of mangrove leaves in hypoglycemia. Mice blood were drawn as followed: a mouse is put into a 50 ml plastic container with the mouse's tail expose to the outside, use cotton with 70o antiseptic, use a blood extract needle to draw blood in the mouse's tail vein (Parasuraman *et al*., 2010) [26].

White mice kidney collection method

White mice are immobilized by stretching the dorsal vertebrae. White mice were operated on and obtained kidneys according to the instructions of Tran Thanh Tong (2000).

Step 1: Prepare a white mice for the operation.

Step 2: Cut the skin and muscle layer.

Step 3: Carefully remove the mouse kidney.

Step 4: Soak the kidneys in formol 10%.

H&E staining method

Hematoxylin-Eosin (H&E) stained sample template (Cardiff *et al*., 2014; Kiernan and Kumar, 1996; Ministry of Health, 2013) [5, 27, 17]. Blue to dark blue cells; pink to red cytoplasms; dark pink erythrocytes; light pink colloidal fibers. The histological sample was read under a microscope, comparing, contrasting and assessing the extent of the lesions of the white mouse nephropathy.

Data processing method

The recorded figures are processed using Sigma Plot statistical software. The average figures are presented in the form of ± SD. $\bar{X}$ ± *SD*. The significance level used to verify the difference in significance between solutions is 0.05 (p<0.05), the difference is statistically significant.

Results

Table 1: Hypoglycemic effect of mangrove plant leaves juice on diabetic rats

Experience	Fasting Blood Sugar (mg/dl) Timelines			
	Day 1	Day 3	Day 5	Day 7
CT1	238, 40±0, 73*	239, 04±0, 27*	239, 35±0, 51*	2400, 16±0, 61*
EX1	238, 22±0, 67*	234, 46±0, 70*	229, 49±0, 42*	225, 39±0, 38*
EX2	238, 10±0, 58*	233, 61±0, 36*	226, 15±0, 60*	219, 16±0, 47*
EX3	238, 00±0, 38*	229, 09±0, 34*	218,67±0, 44*	201, 55±0, 33*
EX4	238, 12±0, 46*	219, 31±0, 26*	199, 52±0, 46*	178, 36±0, 52*
CT2	238, 16±0, 66*	215, 26±0, 28*	185, 34±0, 63*	160, 18±0, 29*

* The difference is statically significant with $p<0.05$

At the beginning of the experiment to determine the ability to lower blood sugar of mangrove-wood leaves on diabetic rats, on the first day, the blood glucose values of the treatments ranged from 238.00 mg/dl to 238.40 mg/dl were similar and there was no statistically significant difference. In the CT1 group, the mice had diabetes but were not treated with mangrove-wood or specialized hypoglycemic drugs, so in 7 days, glucose levels ranged from 238.36 mg/dl to 240.16 mg /dl, there was no hypoglycaemia, the blood glucose remained high, indicating that the CT1 mice remained diabetic. Untreated rats experience hyperglycemia (which often develops in type 2 diabetes) when there is an imbalance in hepatic glucose production during fasting and in blood glucose (Lee and Halter, 2017) [15]. In general, in the cages of mice treated for diabetes, the majority of blood glucose levels were lowered. After 7 days of experiment, batches of EX1, EX2 and EX3 all had a decrease in blood glucose concentration. The glucose content of mice in EX1 decreased by 12.83mg/dl, in EX2 decreased by 18.94mg/dl, in EX3 decreased by 36.45 mg/dl. But the result is 225.39 mg/dl respectively; 219.16 mg/dl and 201.55 mg/dl both had values greater than 200 mg/dl, indicating that the rats in these treatments were still diabetic (Kim, 2012) [12]. In EX4 with a concentration of 400mg/kg mangrove-wood extract, it showed good therapeutic potential when after 7 days the blood glucose concentration of diabetic white mice decreased to 59.76 mg/dl to 178.36. mg/dl (<200 mg/dl) showed that rats in EX4 lower blood sugar better than other treatments. For CT2, using Diabetna, which is a drug with hypoglycemic and anti-diabetic effects, showed that diabetic white mice were treated and lowered blood sugar, when after 7 days, blood glucose levels were valuable. Value 160.18 mg/dl. All of those differences are statistically significant with $p<0.005$.

2. Effect of mangrove plant leaves juice on diabetic rat kidney

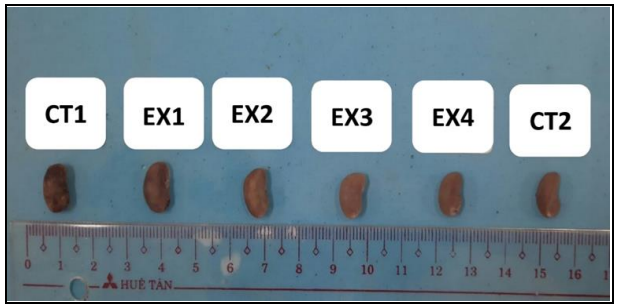

Fig 1: Renal morphology of white mice in experiments

When observing the external morphology of the white mouse kidney, a clear difference can be seen with the naked eye. The results in CT1 were that untreated diabetic rats had larger kidneys (Mogensen and Andersen, 1973) [18] than the controls, rough surface, cyanotic color, fibrous surface with many ulcers and tumors. Evaluated for the degree of diabetic kidney cancer (Gharaibeh *et al*., 2022) [19]. Compared with CT1, in EX1 the kidney surface is rough, fibrous, less ulcerated, there is a hematoma on the surface of the kidney. Compared with the EX1 kidney, the surface of EX2 and EX3 is smooth, and there are ulcers and bruises on the kidney surface. In EX4 and CT2 the surface is smooth, morphologically similar to that of a normal white mouse kidney, with no other signs of the kidney surface.

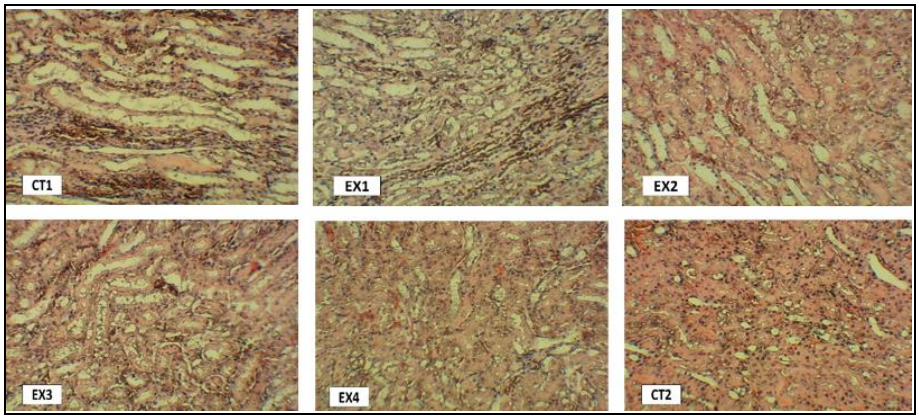

Fig 2: H&E staining pattern of kidney tissue cells of white mice

When studying, histological analysis of white mouse kidney on H&E staining slides showed the difference between the structure of white mouse kidney tissue between experimental treatments. Through the results of renal tissue staining at CT1, the tissues with structural changes, with many renal fibrotic spaces and renal fibrotic spaces all have a large area, congestion and hemorrhage, besides white blood cells. Lymphocytes were concentrated in one place, showing that around the kidney tissue formed foci of acute nephritis. EX1, EX2 and EX3 on H&E staining slides appear structural

changes, there are many fibrotic spaces, but these fibrous spaces have few and smaller areas compared to CT1, white blood cells are concentrated mainly around the tissue. Renal striations, showing that the interstitium has inflammatory foci that need to be treated, the occurrence of congestion and bleeding around the kidney tissue decreases gradually from EX1 to EX3, demonstrating that the increased dose of mangrove plant leaves juice used will to limit kidney congestion and bleeding in diabetic rats. In EX4 and CT2 there are still some renal fibrosis but very few and very small area. Lymphocytes are scattered, there is no abnormal concentration, the structure of the treated kidney tissue gradually returns to a normal state.

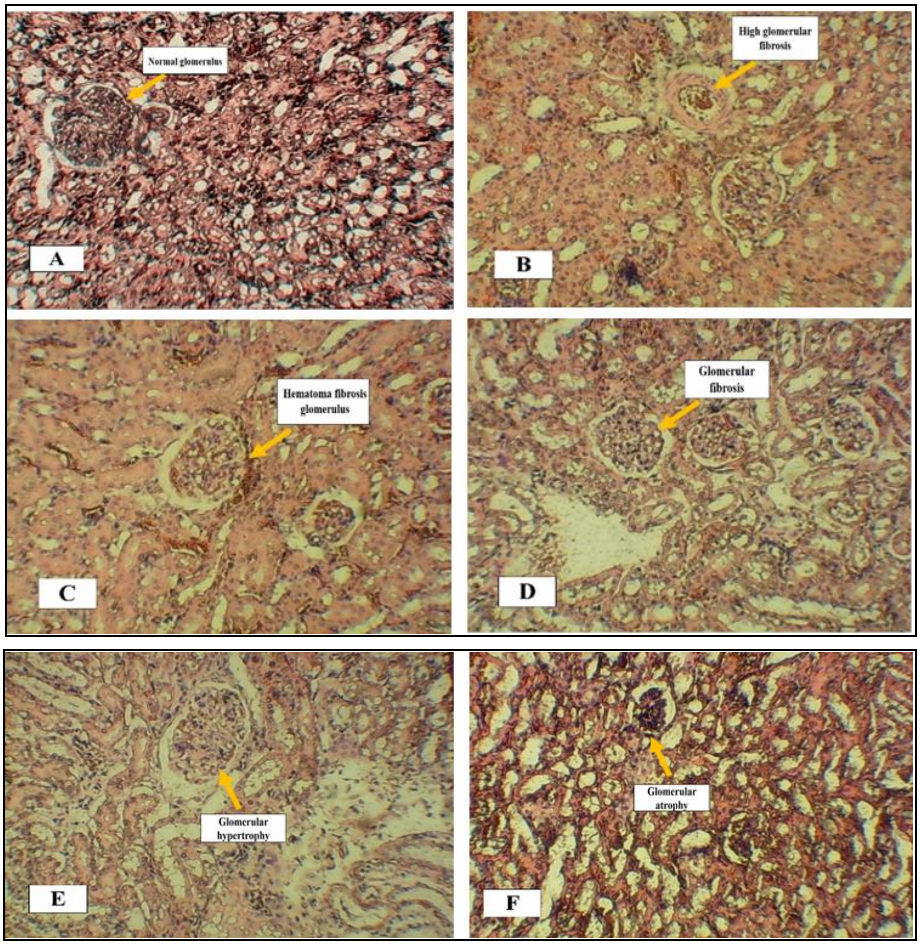

Fig 3: White mice glomerular shape on H&E template

When white mice develop diabetes, three main changes appear inside the kidneys:

First

The vascular organization of the kidneys is dilated; this is a consequence when blood glucose levels in the kidneys rise in excess.

Second

The bottom of the glomeruli becomes thicker, after which the veins of the kidneys gradually expand.

Third

Glomerular fibrosis is formed due to pressure inside the glomeruli, the main reason is that the arterioles are dilated when hyperglycemia causes damage, anemia narrows blood vessels, thereby leading to anemia feeding glomerular cells, many cells die at the glomeruli (Min *et al*., 2012) [16].

After the glomeruli loses its function, it leads to glomerular atrophy. When the team studied and observed the H&E staining pattern - the kidney staining sample of white mice, the team noticed the appearance of different glomerular forms such as: normal glomeruli (glomerular form A), complete fibrous glomeruli (glomerular morphology B), glomerulonephritis with hematomas (glomerular morphology C), partial fibrous glomerulonephritis (glomerular morphology D), glomerular hypertrophy (glomerular morphology E), glomerular atrophy (glomerular morphology F) (Kimmelstiel and Wilson, 1936) [13].

Each nephron contains a glomerular capillary beam called a glomeruli, through which a large amount of fluid is filtered from the blood, and a long tube in which the filtered fluid is metabolized into urine. Each human kidney contains about 800,000 to 1,000,000 nephrons, each of which is capable of forming urine (Bertram *et al*., 2011) [3]. The kidneys are unable to regenerate new nephrons (Becherucci *et al*., 2018) [2]. Therefore, with kidney injury, disease, or normal aging, the number of nephrons gradually decreases causing impaired kidney function. Because a kidney has many glomeruli, the study assessed the frequency of more or less occurrence of glomerular forms.

Glomerular morphology A

In the study only glomerular morphology A was found in EX4 and CT2. In particular, in EX4 there is a greater frequency of occurrence than in CT2. Showed that glomeruli in the normal state were most concentrated in the staining specimens at EX4.

Glomerular morphology B

In the completely fibrous glomerular study found only in CT1, other studies did not have the presence of completely fibrous glomeruli in the dyed samples. Showing that complete fibrous glomeruli is only present in untreated CT1, this is also the basis for confirmation that in CT1 kidney mice are showing signs of kidney cancer.

Glomerular morphology C

In the study appeared in all forms. CT1 and EX1 have the most hematoma-related glomerular occurrences, with statistically equivalent numbers. EX2, EX3 and EX4 have a gradual decrease in the frequency of occurrence of glomerular morphology C, in CT2 there is the least occurrence. When the kidneys have diabetes, it is possible that whether treated or not treated, the kidneys after treatment still have an inflammatory form, and there are inflammations around the kidneys so there is a hematoma around the glomeruli.

Glomerular morphology D and glomerular morphology E

In the study, the frequency of occurrence gradually decreased from CT1, EX1, EX2, EX3, EX4 and CT2. Partial renal fibrosis and glomerular hypertrophy are the basic manifestations of glomeruli with diabetes mellitus D'Agati *et al*., 2011) [6]. Mice in the studies all had diabetes, so in the tests there were two forms of glomeruli.

Glomerular morphology F

In the study found the appearance in CT1, EX1, EX2. With the most frequency of CT1 occurrence and higher occurrence in EX1 than EX2. Indicates glomerular atrophy, loss of kidney function is occurring at CT1, EX1 and EX2.

Analysis of results

The results in this study showed that the fasting blood glucose of white mouse blood was reduced when mangrove plant leaves fluid was used. On the fourth day, EX4 had a greater decrease in glucose levels than CT2, indicating that the hypoglycemic effect of syrup extract after 4 days was more effective than that of specialized Diabetna. After 7 days, CT2 performed better than EX4. Other tests in this study also showed lower blood glucose levels.

Long-term diabetes mellitus is often accompanied by glomerulonephritis and eventually kidney failure and kidney cancer (Larsson and Wolk, 2011) [14]. Histologically, kidney cancer only develops from previously visible and existing kidney damage that we do not know about (Larsson and Wolk, 2011) [14], in the CT1 study when diabetic kidneys found tumors on the surface of the kidneys. The study showed that histopathological images from diabetic kidneys clarified that mangrove plant leaves fluid has the ability to protect kidney tissue and glomeruli from damage, limiting the level of kidney cancer caused by diabetes mellitus.

Conclusion

A model of diabetic mice treated with syrup (*Acanthus ilicifolius*) initially showed a decrease in blood glucose levels after 7 days of treatment. Renal manifestations of diabetic white mice were markedly effective among the studies. Similarly, histopathology also shows that mangrove plant leaves fluid has the ability to treat and protect the kidneys from nephritis pathogens. In some of the factors shown in EX4 there is a better therapeutic effect than CT2.

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