ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
Index Copernicus Value: 49. 23
PubMed - National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 4, Issue – 2, March - 2019, Page No. : 177 - 182

The Potential Hypoglycemic Activity of Pinus merkusii Bark Ethanolic Extract in Streptozotocin-Induced Diabetic Rats

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Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

In this study, in vivo hypoglycemic activity of Pinus merkusii bark extracts was evaluated against streptozotocin-induced diabetic rats. The rat of male were divided into control group (Rats were given with distilled water); diabetic group (Rats were injected with streptozotocin 65 mg/kg BW i.p), and the treatment group (Rats were injected with streptozotocin 65 mg/kg BW i.p and were given the *Pinus merkusii* extract 200 mg; 400 mg; 800 mg/kg BW orally). The blood were taken to be measured glucose, insulin level. Furthermore, rats were sacrificed and pancreas tissues were used for the analyzes of MDA. Administration of streptozotocin resulted significantly in increased glucose and MDA the level, and also decrease in the level of insulin were compared with the control group. Administration of the *Pinus merkusii* extract only 800 mg/kg BW significant decreased the glucose and MDA levels, and also increase in the level of insulin. It could be concluded that, the potential of the *Pinus merkusii* extract may be useful as a hypoglycemic agent in against streptozotocin-induced diabetic rats

Keywords: Pinus merkusii, Hypoglycemic, MDA, Insulin

Introduction

Diabetes mellitus (DM) is the most common endocrine disorder resulting from a defect in insulin secretion, insulin resistance or both. It is the third leading cause of morbidity and mortality, after heart attack and cancer [1,2]. In general, DM is classified into two categories: type 1 and type 2. In type 1 diabetes (T1DM), hormone insulin is not produced due to the destruction of pancreatic β cells, while type 2 diabetes (T2DM) is characterized by a progressive impairment of insulin secretion by pancreatic β cells and by a relative decreased sensitivity of target tissues to the action of this hormone [3,4]. Chronic diabetes melitus causes many complications, including nephropathy, retinopathy, neuropathy, and macrovascular and microvascular damage [5].

Nowadays, different treatments, such as insulin therapy, pharmacotherapy, and diet therapy, are available to control diabetes. There are several types of glucose-lowering drugs that exert anti-diabetic effects through different mechanisms. These mechanisms include

stimulation of insulin secretion by sulfonylurea and meglitinides drugs, increasing of peripheral absorption of glucose by biguanides and thiazolidinediones, delay in the absorption of carbohydrates from the intestine by alpha-glucosidase, and reduction of hepatic gluconeogenesis by biguanides [5,6]. In the past three decades, despite the significant progress made in the treatment of diabetes, the results of treatment in patients is still far from perfect. These treatments have some disadvantages, including drug resistance (reduction of efficiency), side effects, and even toxicity [3,4]. Besides conventional oral and injectable medications, diabetes treatments include diet modification, regular exercising, lifestyle changes, weight regulation and other alternatives or add on therapies such as herbal therapy. Herbal drugs are prescribed widely as drugs of choice because of their effectiveness, few side effects and relatively low cost [7,8]. Today, many treatments that involve the use of medicinal plants are recommended. The anti-hyperglycemic effects that results from treatment with plants are often due to their ability as antioxidant to improve the performance of pancreatic tissue, which is done by increasing insulin secretions or reducing the intestinal absorption of glucose [9,10].

It has been demonstrated that *Pinus* plant has phytochemicals including alkaloids, polyphenols, flavonoids, lignans, triterpenes, sterols, glycosides, triterpenoids, and saponins [11,12]. Recent research activities have shown that *Pinus* plant are potent as an antioxidant, antibacterial agents, antiallergic, antiinflammatory, cardioprotective, immune-stimulating, antiviral, and antidiabetic activities [13,14,15]. The objective of the present study was to investigate the hypoglycemic activity of the *Pinus merkusii* extract on streptozotocin-induced diabetic in Wistar albino rats.

Materials & Methods

Experimental Animal

Male Wistar rat weighing approximately 200–250 g (2.5–3 months) were obtained from the Gadjah Mada University, Yogyakarta, Indonesia, for experimental purpose. They were housed in plastic cages in an air-conditioned room with a temperature maintained at 26 ± 2°C and 12 h alternate light and dark cycles. The rats were given *ad libitum* with tap water and fed with a standard commercial rat. This study was reviewed bythe Ethical Clearance Committee for preclinical research, Faculty of Dentistry, Airlangga University

Induction of Diabetes

Streptozotocin (STZ)-induced diabetes has been described as a useful experimental model to study the activity of hypoglycemic agents. After an overnight fasting (deprived of food for 16 hours had been allowed free access to water), diabetes was induced in rats by intraperitoneal injection of STZ (Sigma, St. Louis, Mo) dissolved in 0.1M sodium citrate buffer pH 4.5 at a dose of 65 mg/kg body weight (22). The control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After a week time for the development of diabetes, the rats with moderate diabetes having glucosuria and hyperglycemia (blood glucose range of above 250 mg /dl) were considered as diabetic rats and were used for the further experiments.

Plant Material Collection and Preparation of Extracts

Pinus merkusii bark were collected from Pacet, Mojokerto, East of Java, Indonesia. The dried bark were powdered using a milling machine. The powder, weighing about 1 kg, was sequentially extracted by maceration with 3 liter ethanol (80%) for 4 days. The extracts obtained

were filtered with Whatman No.1 filter paper and concentrated in vacuo by a rotary evaporator at reduced pressure.The concentrated extracts were dried in the oven (50 °C) to remove the remaining solvents and the dried extracts were kept in the freezer (−25 °C) until further use in the designated experiments

Experimental Design

The sample used 50 male rats were divided into control group (Rats were given with distilled water) ; Diabetic group (Rats were injected with streptozotocin 65 mg/kg BW i.p), and the treatment group (Rats were injected with streptozotocin 65 mg/kg BW i.p and on day 4, were given the *Pinus merkusii* extract 200 mg; 400 mg; 800 mg/kg BW orally for 28). On day 32, the rats blood samples were taken by cardiac puncture to be measured levels of glucose and insulin. Blood glucose was measured using a glucometer (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). The levels of Serum insulin were determined using an Insulin ELISA Assay Kit.[5] Furthermore, rats were sacrificed and pancreas tissues were used for the analyzes of MDA. MDA was determined in the supernatant of homogenate pancreas tissue by the thiobarbituric acid method. MDA is expressed as nanomoles MDA/g tissue.

Results

Effects of Pinus merkusii Extract on Streptozotocin-Induced Changes in the Blood Glucose and Serum Insulin.

An increase in the blood glucose and decrease serum insulin suggests indicated pancreas damage. Analysis of these blood glucose and serum insulin have been done to evaluate the *Pinus merkusii* extract on streptozotocin treated rat. The diabetic group showed significantly (p <0.05) increase in blood glucose and decrease serum insulin were compared with the control group. In contrast, the groups pretreated with *Pinus merkusii* extract (only at dose 800 mg/kg BW) showed significantly (P <0.05) decreased blood glucose and increase serum insulin in a dose-dependent manner were compared with the diabetic group and close to the control group (Table-1).

Table-1: Pinus merkusii extract nanoparticles on streptozotocin-induced changes on the blood glucose and serum insulin

Groups	Means ± SD	
	Blood Glucose (mg/dl)	Serum Insulin (µU/ml)
Control	108.21^{a} ± 9.23	78.63^{a} ± 4.81
Diabetic groups	286.17^{b} ± 14.43	51.32^{b} ± 5.83
Pinus merkusii extract 200 mg/kg BW	278.31^{b} ± 18.67	49.51^{b} ± 6.24
Pinus merkusii extract 400 mg/kg BW	268.62^{b} ± 13.92	58.24^{b} ± 4.64
Pinus merkusii extract 800 mg/kg BW	158.57^{c} ± 11.23	62.37^{c} ± 3.95

Superscript within each column indicates significant difference between the means ($p < 0.05$)

Effects of Pinus merkusii Extract on Streptozotocin-Induced Changes on MDA in Rat Pancreas

The streptozotocin enhances the intracellular formation of reactive oxygen species causing pancreas damage. In the present study, we analyze the pancreas levels of MDA. The diabetic group showed significant (P< 0.05) increase in the level of MDA were compared with control group. In the groups pretreated with *Pinus merkusii* extract (at dose 800 mg/kg BW but not 200 mg/kg BW and 400 mg/kg BW) showed a significant (P <0.05) decrease MDA were compared with diabetic group and close to the control (Table-2).

Table-2: Effects of *Pinus merkusii* extract on streptozotocin-induced changes on MDA in pancreas

Groups	MDA (nmol/mg)
Control	$8.29^{a} \pm 0.81$
Lead acetate groups	$13.35^{b} \pm 9.12$
Chitosan-P.merkusii 150 mg/kg BW	$12.42^{b} \pm 0.83$
Chitosan-P.merkusii 300 mg/kg BW	$11.87^{b} \pm 0.93$
Chitosan-P.merkusii 600 mg/kg BW	$10.11^{c} \pm 0.66$

[a,b,c]Different superscript within each column indicate significant difference between the means ($P < 0.05$)

Discussion

Diabetes mellitus is a metabolic disorder involving oxidative stress, which induces insulin resistance in the peripheral tissues and impairs insulin secretion by pancreatic β-cells [10,16]. A large number of hypoglycaemic (antidiabetic) plants and herbs have been studied by modern methods and are starting to be introduced into modern therapy. Streptozotocin (STZ), a structurally glucosamine derivative of nitrosourea, has been widely used for inducing experimental diabetes mellitus in rats. STZ impairs glucose oxidation and decreases insulin biosynthesis and secretion [17,18].

Our results in this study found that a significant increase in blood glucose and a decrease in serum insulin levels were showed after treatment rat with streptozotocin. This effect might be related to the oxidative stress plays a crucial role in inducing pancreatic islet β-cell injuries and probably as a result of excessive levels of mitochondrial ROS production and the presence of fewer antioxidant enzymes in pancreatic β-cells. Streptozotocin produces oxidative damage in the pancreas by enhancing lipid peroxidation and cause pancreas dysfunction and increase free radical damage [17,18]. The administration of *Pinus merkusii* extract 800 mg/kg BW showed an improvement in the blood glucose and insulin levels. This might be through its direct action on free radicals of streptozotocin to prevent the pancreatic cellular damage by maintaining its membrane integrity. It has been reported that *Pinus* plant has phytochemicals including alkaloids, polyphenols, flavonoids, lignans, triterpenes, sterols, glycosides, triterpenoids, and saponins [11]. Recent research activities have shown that *Pinus* plant are potent as an antioxidant [19]. The antioxidants have been suggested to afford protection to the pancreas against oxidative stress in diabetes mellitus. In present study, administration of streptozotocin also can increase MDA and inducing oxidative damage in the pancreas. MDA may be used as an indicator of cell membrane injury. The increase in MDA levels in pancreas suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Treatment of rats with *Pinus merkusii* extract at a dose of 800 mg/kg BW decrased the levels of MDA to rise when the rats were challenged with streptozotocin in the pancreas, which might be due to the ability of *Pinus merkusii* extract nanoparticle to reduce the accumulation of free radicals. It is known that *Pinus merkusii* extract, which behaves as a powerful antioxidant and free radical scavenger, can decrease the MDA level perturbed by streptozotocin in rats pancreas [10,16]. The findings of this study suggest that Pinus merkusii extract could attenuate oxidative stress which may inhibit in β-cell deterioration by decreasing the lipid peroxidation (MDA level). Therefore Pinus merkusii can increase insulin secretion.

Conclusion

In conclusion, the present study indicates that the *Pinus merkusii* extract is a natural product that contains potent antioxidant compounds and presents good prospects for the improve the effects of streptozotocin-induced diabetic rat. Therefore, Pinus merkusii can be considered as a plant with enormous potential as an anti-diabetic agent; however, the investigation of its major active constituents is still in progress.

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