AMELIORATIVE EFFECT OF PLANT EXTRACTS OF SURUHAN (PEPEROMIA PELLUCIDA) ON BLOOD GLUCOSE AND LIBIDO OF MALE MICE INJECTED WITH ALLOXAN

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INTRODUCTION

Patients suffering from diabetes mellitus (DM) disease, due to hyperglycemic condition, will experience oxidative stress and pathophysiological disorders that are well known as the risk factors to accelerate the onset and progression of various complication among diabetic patients.[1] In man, such the carbohydrate metabolism-related disorder can causes sexual dysfunction and infertility. Diabetes-related sexual dysfunction may closely related to lower testosterone levels and be manifested in all its forms such as reduced erection, impotence, and other libido dissociations.[2, 3]

In addition diabetes has also known to cause dysregulation of epigenetic modification during spermatogenesis leading to the decrease in sperm motility, sperm DNA integrity, and ingredients of seminal plasma.[4]

One of the standard drugs that is widely used to treat diabetes mellitus is glibenclamide. However, the use of this drug is not free from side effects.[5] Therefore, the search for safe plant-based medicines is still continues. Among traditional people of South East Asian region, one type of plants that has commonly used as anti-diabetes herbs is *Peperomia pellucida* L. Kunth. that in Indonesia locally called suruhan.[6] However, the effect of the plant herbs of suruhan on sexual function and fertility of male subjects is not yet known.

This study aims to determine whether the extract of *Peperomia pellucida* plant which was believed to be efficacious for treating diabetes can ameliorate other pathophysiological defects related to diabetes, especially those related to fertility and sexual function in male subjects.

MATERIAL AND METHODS

Plant Materials

Whole plant samples of suruhan (*Pepromia pellucida* L. Kunth) used in the study were collected from suburb of Bandar Lampung, Indonesia. The weeds were washed with aquadest, air dried, sliced into small pieces, and then soaked in 96% ethanol for 24 hours. After being macerated for four times, the macerate evaporated using rotary evaporator under low pressure at 50°C until brownish-viscous extract formed.

Animals and Experimental Design

In this study, male albino mice aged 3-4 months, weighing between 30-40 g, obtained from Lampung Veterinary Center, Bandar Lampung, Indonesia were used. The animals were handled according to the Ethical Clearance from Faculty of Medicine, University of Lampung, Indonesia. They were maintained under room

temperature, fed with a standard laboratory diet and water *ad libitum*. By using a completely randomized design, 25 male albino mice were grouped into five with five replications each. Group 1 treated with alloxan at the dose of 150 mg/kg bw (as negative control). Group 2 was given alloxan and glibenclamide of 0.65 mg/kg bw (as positive control). Group 3, 4 and 5 were treated with alloxan and plant extracts at the dose of 56, 112 and 168 mg/kg bw respectively.

Extract Administration

In this experiment test animals were conditioned to experience hyperglycemia. The hyperglycemic condition of experimental animals was made by intraperitoneally injecting 0.5 ml of alloxan monohydrate (Sigma Aldrich, Cat.No.A7413-10G) at the dose of 120 mg/kg body weight after the mice were fasted for 8 hours. Before and after alloxan injection, blood glucose levels of each animal were measured using strip glucometer (from Roche, Germany).

Sexual Behavior Tests

To assess the libido potential of the test animals, all mice that had been treated with/without plant extract of suruhan were mated with estrous virgin females. The tests were carried out in an open round plastic tray with a diameter of 40 cm and height of 25 cm, as implemented by Kanedi et al.,(2015).[7] The tray was divided into two halves, which were separated by a removable cardboard partition. Both males and females subjected to the tests were allowed to adapt to the tray environment with the partition closed for about 5 min.

When the cardboard partition was removed, the mating activities of the mice were observed for 30 min. Throughout the experiment, videotaping was performed to observe the following parameters: courtship latency, mount latency and mount frequency. Courtship latency is the time from when the partition board was opened until the male displayed the first courtship action. Mount latency is the time from when the cardboard was removed until a first mounting action was shown by the males. Mount frequency is defined as the total number of attempts made by the male to ride on the female's back.

Data Analysis

The data, presented as the mean ± SD (standard deviation), were analysed using one-way ANOVA (analysis of variance). When a significant difference was detected by ANOVA, the treated groups were then compared with each other and the control group using the LSD (Least Signicant Diference) test. Differences were considered to be statistically significant when $p < 0.05$.

RESULTS

Blood Glucose Levels

Blood glucose levels of mice before alloxan inducement and after treatment with standard drug and plant extracts of suruhan were presented in Table 1. Based on the statistical analysis it is clear that ethanol extracts of suruhan significantly ameliorate blood glucose levels of mice subjected to hyperglycaemia by alloxan injection close to normal levels.

Table 1: Base line characteristics and treatment effects on blood glucose levels of mice.

Treatment	Blood glucose (mg/dl)
K_0 (base line)	75.96 ± 2.230^{a}
K_- (alloxan 150 mg)	183.60 ± 16.426^{d}
K_+ (alloxan + glibenclamide 0.65 mg)	77.80 ± 3.114^{a}
P_1 (alloxan + suruhan 56 mg)	78.40 ± 5.273^{a}
P_2 (alloxan + suruhan 112 mg)	95.60 ± 7.335^{b}
P_3 (alloxan + suruhan 168 mg)	115.80 ± 7.855^{c}
Data are presented as mean ± SD. Values followed by the same superscripts are not statistically different at $\alpha = 0.05$ by LSD test	

Mating Test

Table 2, 3, and 4 consecutively show effects of plant extracts of *Peperomia pellucida* on courtship latency, mount latency, and mount frequency of male mice mated with normal virgin females. From Table 2 it is revealed that in comparison to negative control group, male mice treated with ethanol extract of suruhan at all levels of concentration show shorter courtship latency.

Table 2: Effects of suruhan extract on courtship latency of male mice in mating test.

Treatment	Courtship Latency (sec)
K_- (alloxan 150 mg)	31.20 ± 7.69^{b}
K_+ (alloxan + glibenclamide 0.65 mg)	18.80 ± 6.01^{a}
P_1 (alloxan + suruhan 56 mg)	14.80 ± 3.92^{a}
P_2 (alloxan + suruhan 112 mg)	22.60 ± 4.77^{a}
P_3 (alloxan + suruhan 168 mg)	16.60 ± 4.39^{a}
Data are presented as mean ± SD. Values followed by the same superscripts are not statistically different at $\alpha = 0.05$ by LSD test	

Next, as shown in Table 3, all treatment groups significantly perform shorter mounting latency. Lastly, Table 4 presents riding trials performed by male on female's back. These data confirm results shown in Table 2 and 3, that plant extract of suruhan tend to show higher sexual drive in male mice compared with that of negative control group

Table 3: Effects of plant extracts of suruhan on mount latency of male mice in mating test

Treatment	Mount Latency (sec)
K_- (alloxan 150 mg)	576.60 ± 52.32^{c}
K_+ (alloxan + glibenclamide 0.65 mg)	185 ± 21.63^{b}
P_1 (alloxan + suruhan 56 mg)	127.40 ± 35.5^{a}
P_2 (alloxan + suruhan 112 mg)	207.20 ± 17.45^{b}
P_3 (alloxan + suruhan 168 mg)	194.80 ± 21.32^{b}
Data are presented as mean ± SD. Values followed by the same superscripts are not statistically different at α = 0.05 by LSD test	

Table 4: Effects of plant extracts of suruhan on mount frequency performed by male mice in mating test.

Treatment	Mount Frequency
K_- (alloxan 150 mg)	0.20 ± 0.44^{a}
K_+ (alloxan + glibenclamide 0.65 mg)	4.80 ± 0.83^{c}
P_1 (alloxan + suruhan 56 mg)	12.00 ± 2.12^{d}
P_2 (alloxan + suruhan 112 mg)	3.40 ± 1.14^{bc}
P_3 (alloxan + suruhan 168 mg)	2.20 ± 0.447^{b}
Data are presented as mean ± SD. Values followed by the same superscripts are not statistically different at α = 0.05 by LSD test	

DISCUSSION

Diabetes is a metabolic disorder related to the defects of insulin secretion and impaired peripheral insulin function and characterized by an increase in blood glucose levels above the normal limit or hyperglycemia. Hyperglycaemia will interfere with the metabolism of glucose, fat, and protein leading to extensive systematic damages.[8]

This study results confirm the efficacy of the Peperomia pellucida plant as an antidiabetes as reported by Hamzah et al.,(2012),[9] and accordingly confirm traditional claims on antidiabetic use of this plant. Our data clearly suggest that plant extracts of suruhan effective in ameliorating blood glucose levels of mice subjected to hyperglycaemic by alloxan injection.

Beside its anti-hyperglycaemic effects, *Peperomia pellucida* (L) Kunth is also reported to possess various pharmacological properties including antipyretic, analgesic, anti-inflammatory, antimicrobial, refrigerant, antioxidant , anti-hyperuricemia, burn healing, depressant, gastroprotective, hypotensive, cytotoxic, lipase inhibitory, fibrinolytic and thrombolytic, anti-diarrhoeal, and anti-osteoporotic.[10-12]

The variety of health benefits of the Peper plant is possible because these plants contain a lot of active ingredients. In addition to mineral elements such as sodium, potassium, calcium, zinc, iron, manganese, lead and phosphorus.[13] *Peperomia pellucida* extracts also contain stigmasterol, analogue of pheophytin and b-sitosterol-D-glucopyranoside, alkaloid, glycoside, reducing sugar, flavonoid, tannin, steroid, terpenoid, α-amino acid, neutral compound, phenolic compound and starch.[14-15] By extracting other species of *Peperomia* from Peru, Lira and colleagues found β-caryophyllene, α-humulene, epi-α-bisabolol, sabinene, cryptone and caryophyllene oxide.[16] It has revealed that, alkaloid, polyphenol, glycoside, flavonoid, anthraquinone, phenolic and saponins are bioactives that showed anti-diabetic effects both in test animals and human.[17]

The most important findings of this study is the recovery of the sexual dysfunction of test mice suffering from hyperglycemia by treatment of ethanol extract of *Peperomia pellucida* plant. The active ingredient of this plant that is thought to play a role in the recovery of libido in hyperglycemic mice is piperine.[18] It has been revealed that black pepper extracts, another type of plant belongs to Piperaceae family containing piperine, effectively increase sexual drive in male mice.[19]

CONCLUSION

Plant extracts of suruhan has revealed to be effective in ameliorating blood glucose levels and sexual drive in male mice subjected to hyperglycaemic by alloxan injection. It suggests that plant extracts of suruhan (*Peperomia pellucida* L. Kunth) is potential to be used as antidiabetic drugs as well as male libido enhancing herbs.

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