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Original Research Article

Plant extracts of Suruhan (*Peperomia pellucida* L. Kunth) ameliorate infertility of male mice with alloxan-induced hyperglycemia

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Abstract

Introduction and Aims: This study was conducted to determine the effect of plant extracts of suruhan (*Peperomia pellucida* L. Kunth) on the blood glucose level and sperm parameters in male mice with alloxan-induced hyperglycemia**.**
Materials and methods: 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. Injection of alloxan was done 3 times in 6 days and the plant extract was given every day for 35 days.
Results: Results showed plant extracts of suruhan significantly lowering blood glucose levels, ameliorate sperms count, viability, motility, and morphology.
Conclusion: It suggests that plant extract of *Peperomia pellucida* is potential to be used as antidiabetic and male fertility recovery agents
Keywords: *Peperomia pellucida*, Suruhan, antidiabetes, sperm parameters, male fertility.

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1. Introduction

Diabetes is a chronic disease due to metabolic problems characterized by a high level of blood sugar or hyperglycemia [1, 2]. Prolonged hyperglycemia may lead to severe damage to the tissue and vascular system leading to serious complications such as neuropathy, cardiovascular complications [3,4]. In addition, diabetes was also indicated to have many indirect effects against most of endocrine function [5]. One of the common implications of hyperglycemia-related neuropathy and vascular disorders among male with diabetes is infertility [6].

Because diabetes-related complications are caused by high blood sugar levels, the strategy to overcome diabetes complications is to reduce blood sugar levels such as using a well known standard drug glibenclamide. The use of this drug, unfortunately, is not free from side effects [7]. That is why the search for safe plant-originated medicinal herbs still going on. Some of medicinal herbs that are known to show anti-diabetic properties are *Momordica charantia* L., *Pterocarpus marsupium* Roxb., and *Trigonella foenum* graecum L.[8]. Among traditional people of South East Asian region, especially in Indonesia, one type of plants that has commonly used as anti diabetic herb is suruhan (*Peperomia pellucida* L. Kunth.) [9].

However, there have been no research reports on the efficacy of suruhan to treat sexual dysfunction in humans and animals suffering from diabetes. This study aims to determine whether the extract of *Peperomia pellucida* plant can ameliorate other pathophysiological defects related to diabetes, especially those related to male fertility.

2. Material and Methods

2.1 Plant materials

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

2.2 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. The animals 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.

2.3 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 three times in six days. Before and after alloxan injection, blood glucose levels of each animal sampled from tail vein, were measured using strip glucometer (from Roche, Germany). All treatment: glibenclamide dan plant extract of suruhan were given orally once daily for 35 days.

2.4 Sperm quality assessment

To assess fertility of animals based on sperm quality parameters sperms count, viability, motility and normal morphology, semen in the epididymis was squeezed and diluted with physiological saline. Spermatozoa was counted using a Neubauer's haemocytometer under a light microscope at 400x magnification and expressed as million/ml of suspension. Quantitative epididymal sperm motility expressed as an index determined by counting both motile and immotile spermatozoa per unit area. Sperm morphology was assessed from a smear of the epididymal filtrate prepared on a clean glass slides by addition of a drop of 1% eosin. After the object dried observation done under a light microscope at 400x magnification and abnormalities of either head or tail were noted.

2.5 Statistical analysis

The data, presented as the mean ± SD (standard deviation), were analyzed 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 Significance Difference) test. Differences were considered to be statistically significant when $p < 0.05$.

3. Results

3.1 Blood glucose levels

Blood glucose levels of mice before (baseline) alloxan injection and after treatments are presented in Table 1. It is clear that without any treatment, blood glucose level of the animals remain highest among groups. All three concentrations of plant extract are significantly reduced blood glucose of mice compared with negative control group. However, the most effective concentration of the plant extract is 56 mg/kg bw.

Table1. Blood glucose levels of mice before (baseline) inducement and after extracts treatments

Treatment	Blood Glucose (mg/dl)
Baseline	75.96 ± 2.23^{a}
Control+ (glibenclamide 0.65 mg)	77.80 ± 3.11^{a}
Control- (alloxan 120 mg/kg bw)	183.60 ± 16.42^{d}
Suruhan (56 mg/kg bw)	78.40 ± 5.27^{a}
Suruhan (112 mg/kg bw)	95.60 ± 7.33^{b}
Suruhan (168 mg/kg bw)	115.80 ± 7.85^{c}

Values are presented as mean ± SD; values followed by the same superscripts are not statistically different at α = 5% by LSD test.

3.2 Sperm parameters

Table 2, 3, 4 and 5 consecutively show effects of plant extract of *Peperomia pellucida* on sperms count, motility, viability and abnormal morphology of mice subjecting hyperglycemia by alloxan injection.

Table 2: Effects of plant extract of suruhan on sperms count in mice with alloxan-induced hyperglycemia

Treatment	Sperm count (10^{6}/ml)
Control+ (glibenclamide 0.65 mg)	31.10 ± 4.43^{b}
Control- (alloxan 120 mg/kg bw)	12.70 ± 2.13^{a}
Suruhan (56 mg/kg bw)	32.90 ± 3.13^{b}
Suruhan (112 mg/kg bw)	30.40 ± 13.29^{b}
Suruhan (168 mg/kg bw)	26.20 ± 3.8^{b}

Values are presented as mean ± SD; values followed by the same superscripts are not statistically different at α = 5% by LSD test.

The data in Table 2 revealed that plant extract of suruhan is significantly ameliorating sperms count of mice compared with that of negative control group. From Table 3 it was found that candlenut extract effectively increased the sperm motility of mice that had hyperglycemia due to alloxan injection.

Table 3: Effects of plant extract of suruhan on sperm motility in mice with alloxan-induced hyperglycemia

Treatment	Sperm motility (%)
Control+ (glibenclamide 0.65 mg)	$52.40 \pm 10.11^{c\ d}$
Control- (alloxan 120 mg/kg bw)	15.00 ± 5.78^{a}
Suruhan (56 mg/kg bw)	60.20 ± 4.65^{d}
Suruhan (112 mg/kg bw)	$41.00 \pm 7.90^{b\ c}$
Suruhan (168 mg/kg bw)	37.00 ± 12.47^{b}

Values are presented as mean ± SD; values followed by the same superscripts are not statistically different at α = 5% by LSD test.

Effects of plant extract of suruhan on sperms viability of mice subjecting hyperglycemia, as shown in Table 4, statistically significant in comparison to the negative control group. Based on the data in Table 5 it is suggested that plant extract of *Peperomia pellucida* significantly maintained sperms morphology in mice with alloxan-induced hyperglycemia.

Table 4: Effects of plant extract of suruhan on sperm viability in mice with alloxan-induced hyperglycemia

Treatment	Sperm viability (%)
Control+ (glibenclamide 0.65 mg)	$52.20 \pm 15.64^{b\ c}$
Control- (alloxan 120 mg/kg bw)	25.00 ± 11.66^{a}
Suruhan (56 mg/kg bw)	67.60 ± 14.57^{c}
Suruhan (112 mg/kg bw)	$37.20 \pm 11.45^{a\ b}$
Suruhan (168 mg/kg bw)	48.20 ± 8.95^{b}

Values are presented as mean ± SD; values followed by the same superscripts are not statistically different at α = 5% by LSD test.

Table 5: Effects of plant extract of suruhan on sperm abnormal morphology in mice with alloxan-induced hyperglycemia

Treatment	Abnormal morfology (%)
Control+ (glibenclamide 0.65 mg)	23.80 ± 5.63^{a}
Control- (alloxan 120 mg/kg bw)	75.80 ± 7.05^{c}
Suruhan (56 mg/kg bw)	$25.20 \pm 10.30^{a\ b}$
Suruhan (112 mg/kg bw)	$31.80 \pm 11.43^{a\ b}$
Suruhan (168 mg/kg bw)	36.20 ± 7.39^{b}

Values are presented as mean ± SD; values followed by the same superscripts are not statistically different at α = 5% by LSD test.

4. Discussion

Peperomia pellucida is a weed plant belongs to Piperaceae family. This plant group is well known for its health benefits due to contain a lot of minerals such as sodium, potassium, calcium, zinc, iron, manganese, lead and phosphorus [10]. A research report from Peru indicated that one species of *Peperomia* contains ß-caryophyllene, a-humulene, epi-a-bisabolol, sabinene, cryptone and caryophyllene oxide [11]. In addition to minerals, *Peperomia pellucida,* revealed to contain many bio-actives such as stigmasterol, analogue of pheophytin and bsitosterol-D-glucopyranoside, alkaloid, glycoside, reducing sugar, flavonoid, tannin, steroid, terpenoid, a-amino acid, neutral compound, phenolic compound and starch [12, 13].

As has been reported by many previously studies, plant species that are commonly suggested to have antidiabetic activities containing bioactives similar to that of *Peperomia pellucida* including flavonoids, saponins, alkaloids, terpenoids, steroids and tannins [15-17].

Besides being proven to be effective in reducing blood sugar levels, plant extracts of suruhan, as shown in Table 2-5, have also been proven to effectively restore male fertility parameters in mice with alloxan-induced hyperglycemia. The efficacy of plant extract of suruhan against the infertility of male mice with hyperglycemia can also be associated with the presence of minerals and bioactives mentioned above. It has been indicated that phenolic compounds (phenols, sterol, lignans and flavonoids, vitamins, folic acid, bio-trace elements (Ca, Mg, P, Zn, K, Cu and Fe), most of the essential amino acids, volatile oils, polyphenols and saponins have positive effects on sperm motility, count, and viability [18].

5. Conclusion

Ethanol plant extract of suruhan (Peperomia pellucida L. Kunth) is proven to be effective in lowering blood glucose and ameliorating sperm parameters in mice with alloxan-induced hyperglyvcemia. It is worthy to conclude that plant extract of Peperomia pellucida is potential to be used as antidiabetic and male fertility recovery agents.

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