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GAVIN PUBLISHERS

Journal of Pharmaceutical and Pharmacological Sciences

Research Article

Nwokike MO, et al. J Pharma Pharma Sci: 4: 187.
DOI: 10.29011/2574-7711.100087

The Effect of *Imperata Cylindrica* Root Aqueous Extracts on Serum Testosterone Levels of Hyperglycemic Rats

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Citation: Nwokike MO, Ghasi SI, Ogbonna AO, Anusiem CA (2020) The Effect of *Imperata Cylindrica* Root Aqueous Extracts on Serum Testosterone Levels of Hyperglycemic Rats. J Pharma Pharma Sci: 4: 187. DOI: 10.29011/2574-7711.100087

Received Date: 29 May, 2020; **Accepted Date:** 08 June, 2020; **Published Date:** 12 June, 2020

Abstract

A decoction of *Imperata cylindrica* root is used by some traditional medical practitioners among the Igbo people for the treatment of erectile dysfunction due to diabetes mellitus but there is no clear scientific evidence is available to back it up. Thus the present study analyzed the effect of an aqueous extract of *Imperata cylindrica* roots on blood glucose and testosterone levels in diabetic Wister rats to establish the pharmacological basis for this folkloric practice.

Methods

Diabetes mellitus was induced by intra peritoneal injection of Alloxan Monohydrate and the diabetic rats were thereafter treated with oral doses of the aqueous extract of *Imperata cylindrica* root once in a day for 28 days consecutively. After the 28th day, rat blood sugar was analysed and serum collected for the analyses of the testosterone level.

Results

The induction of diabetes mellitus led to a decrease in the testosterone level. However, different doses of the extract of *Imperata cylindrica* caused dose dependent increases in the testosterone levels which were found to be statistically significant compared to the control after treatment for 28 days ($P<0.05$ - <0.0001). Conversely, the sugar levels were significantly reduced by the extracts. Phytochemical analyses of the aqueous extract of *Imperata cylindrica* root indicate the presence of carbohydrates and flavonoids.

Conclusion

Flavonoids have been found to possess antidiabetic properties as a result of their modulatory effects on blood sugar transporter by enhancing insulin secretion, reducing apoptosis and promoting proliferation of pancreatic β-cells, reducing insulin resistance, inflammation and oxidative stress in muscle. They have also been found to be involved in the onset of penile erection and improved sexual performance.

Keywords: Diabetes Mellitus; *Imperata Cylindrica*; Testosterone Levels; Wistar Rats.

INTRODUCTION

Testosterone [$C_{19}H_{28}O_2$] is a male steroid hormone produced in the testicles. It is also produced synthetically for treatment of androgen deficiency [1]. Testosterone plays a role in the development of male sex organs and, at puberty, it is involved in developing secondary sex characteristics such as the growth of body hair and facial hair, the deepening of the voice, and increased penis and testes size [2]. In addition to its role in the development of male characteristics, testosterone is also involved in sperm production and sex drive [2]. Low testosterone levels have been associated with erectile dysfunction, reduced sperm count, loss

of muscle mass or hair, reduced sex drive, and increased breast size [3]. Approximately 10 percent of all men experience erectile dysfunction, at some time in their lives and this is attributable to low testosterone [4]. Interestingly the largest group of people who have low testosterone are diabetics [3]. Diabetes is a growing epidemic, with 328 million people worldwide having diabetes and this number is expected to rise to 592 million by the year 2030 [*5*].

Testosterone replacement therapy [TRT] or hormone replacement therapy [HRT] is often used to supplement the natural testosterone production [6]. Other treatment options include medications, topical gels, patches, and injections [6]. Though testosterone replacement therapy may boost testosterone levels, it is generally only recommended as a short-term treatment because it comes with a high risk of side effects. It has been established that testosterone replacement therapy can actually reduce sperm count and these effects could be permanent [7]. Short-term side effects of TRT may include increased appetite, changes in mood, nausea, and vomiting. In addition to reduced sperm count or permanent sterility, some of the long-term effects of TRT may include Liver problems, Prostate tissue growth and/or tumours, Fluid retention, shrinking of the testicles and Permanent dependence on TRT [8-10]. Evidence shows that approximately one third of type 2 diabetics are testosterone deficient. An even greater proportion of men who are both diabetic and obese experience testosterone deficiency, and the likelihood of testosterone deficiency increase as type 2 diabetes progresses or worsen [11-13]. In the late 1990s sildenafil, a drug that treats erectile dysfunction became available by prescription. After its introduction, many men who had previously remained silent about their condition sought treatment [4]. The numerous side effects of current therapeutic interventions for low testosterone, diabetes mellitus and erectile dysfunction led to this search for better treatment options from the plant resource bank for medicine and pharmacology through traditional medical practitioners [14]. It has been estimated that between 60-90% of the populations of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare [15].

Aqueous extracts of *Imperata cylindrica* [AICR], commonly known as spear grass in English or Ata in Igbo languages have been used for the treatment of erectile dysfunction due to diabetes mellitus by traditional healers in Eastern Nigeria. The roots are pleasant to chew and fairly sweet and the taste is sweetest in the wet season. The roots are traditionally used in urinary calculi, retention of urine, diabetes mellitus, cardiac disorders, gout, impotency, cough and cold [16].Numerous studies have been carried out to find the potential of *Imperata cylindrica* for medicinal use [17-20].

Previous Phytochemical studies isolated a lignan glycoside, impecyloside, from the rhizome. The Rhizomes have also been shown to contain arundoin, cylindrin, fernenol, cylindol, cylindrene, graminones and imperanene [21]. The Methanol extract of aerial parts yielded tannins and saponins [22]. But of most importance is that no acute and sub chronic toxicities have been found in rats administered with *Imperata cylindrica* root extracts [23]. Most of the associated medicinal uses of *Imperata cylindrica* roots in Eastern Nigeria are not backed by scientific studies. Thus the present study bridges the knowledge gap on the scientific justification for the use of aqueous extract of *Imperata cylindrica* root by traditional medical practitioners with emphasis on the effect on blood sugar and serum testosterone levels.

MATERIALS AND METHODS

Collection of Plant Material

Imperata cylindrica roots were harvested from Awha Imezi in Ezeagu Local Government Area of Enugu State and were identified and authenticated by Mr. Chijioke John Onyeukwu of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka [UNN]. Voucher specimens are deposited in the International Center for Ethno-medicine and Drug Development herbarium with number UNH166.

Chemicals and reagents

Alloxan monohydrate and Glybenclamide was purchased from Bristol chemicals Lagos, Nigera. The glucometer [ACCU-CHEK Active. Roche Diagnostics GmbH], Enzyme-Linked Immunosorbent Assay [ELISA] Kit [Abcam] and other chemicals and reagents were purchased commercially from local vendors and were of analytical grade.

Preparation of *Imperata cylindrica* root extract

Imperata cylindrica extracts were prepared as previously described by Jayalakshmi *et al.* [24] to resemble as closely as possible the way it is used by traditional medical practitioners [25].

Experimental Animals

A total of two hundred adult male Wistar rats weighing between 150 and 200grams were obtained from the animal house of the Department of Pharmacology and Therapeutics, University of Nigeria College of Medicine. The animals were kept in cages in the animal house for one week to allow for acclimatization with free access to standard animal diet and water *ad libitum*. The research project was reviewed and approved by the University of Nigeria Teaching Hospital Health Research Ethics Committee with No. NHREC/05/01/2008B-FWA00002458-1RB00002323.

Induction of Diabetes Mellitus in Wistar Rats with Alloxan

Alloxan was used for induction of experimental diabetes [26]. Eighty male adult rats weighing 150–250 grams were used for this study. The rats were fasted prior to injection for 10 hours. A single dose of Alloxan monohydrate, 150 mg/kg was administered

as a 5% w/v in distilled water injected intraperitoneally to the rats. Each rat was placed back into the cage after injection. This procedure was repeated for all the animals. The rats were supplied with 10% sucrose water to avoid sudden hypoglycaemia post-injection.

Rats were tested for sufficient levels of hyperglycaemia at 4 weeks post injection. Tail blood samples were obtained from fasting rats for blood glucose levels measurement.

Biochemical Analysis

Forty eight rats were selected for the study: forty with blood glucose levels above 200mg/dl and eight normal rats in which diabetes mellitus were not induced. These were placed into six groups of eight rats in each group:

Group A [Normal Control: Non-Diabetic Rats Treated With Distilled Water],

Group B [Diabetic Control: Alloxan-Induced Diabetic Rats Treated With Distilled Water],

Group C [Test Group1: Alloxan-Induced Diabetic Rats Treated With AICR [200mg//Kg]],

Group D [Test Group2: Alloxan-Induced Diabetic Rats Treated With AICR [400mg/Kg]],

Group E [Test Group3: Alloxan-Induced Diabetic Rats Treated With AICR [600mg/Kg]],

Group F [Positive Control: Alloxan-Induced Diabetic Rats Treated With Glibenclamide [0.5 Mg/Kg]].

The plant extracts/standard drugs were administered by gastric lavage to the rats once in a day for 28 days consecutively.

They were kept in cages separately and their body weight was measured at the beginning and on the 28th day of the study. Twenty four hours after the last *treat*ment blood was collected by cardiac puncture from each animal into clean dry test tubes. The blood samples were allowed to stand for about 30 minutes to clot. Serum was separated from the clot with Pasteur pipettes into sterile sample tubes.

Effect of *Imperata Cylindrica* Roots Aqueous Extract on Blood Glucose Level of Wistar Rats.

Blood samples for this analysis were collected via tail vein-with heparinised capillary tubes, and 25-µL aliquots were used to determine the glucose level. Blood glucose concentrations were determined using a glucometer [ACCU-CHEK Active. Roche Diagnostics GmbH].

Effect of *Imperata Cylindrica* Roots Aqueous Extract on Blood testosterone Level of diabetic Wistar Rats.

Testosterone was assayed using an Enzyme-Linked Immunosorbent Assay [ELISA] Kit [Abcam]. This ELISA kit is designed for the accurate quantitative measurement of Testosterone in serum [27].

Statistical Analysis

Values are expressed as mean ± standard error of mean [SEM] of all the observations. Student t-tests were performed to compare the results obtained from each extract with the controls and then analysed by one-way analysis of variance. Differences among groups were considered as statistically significant at $P<0.05$.

RESULTS

Effect of *Imperata Cylindrica* Roots Aqueous Extracts on Blood Glucose Levels of Alloxan Induced Diabetic Wistar Rats.

The anti-hyperglycaemic effect of the three doses of aqueous extract of *Imperata cylindrica* root on the fasting blood sugar levels of diabetic rats is shown in table 1. Administration of Alloxan [150 mg/kg, i.p.] led to an elevation of fasting blood glucose [FBS] levels to 211.60±2.34 compared to the mean initial FBS value of 87.25±4.01 for the normal non-diabetic control group. The elevated blood glucose was maintained throughout the duration of the study but was reversed after four weeks of daily treatment with *Imperata Cylindrica* roots aqueous extract by 25%–50% for the three analysed doses. The hypoglycaemic activity of the aqueous *Imperata cylindrica* root extract was found to be dose-dependent up to 400mg/kg body weight [204.4±2.61 – 132.6±4.76 and 212.6±3.93 – 117.5±7.69] respectively. The higher dose of 600mg/kg, although showing statistically significant reduction of the fasting blood glucose [217.6±3.27 – 151.5±1.05], failed to reduce the level as much as was observed in the case of the 400mg/kg dose. The positive control 0.5mg/kg glibenclamide showed very statistically significant reduction in fasting blood glucose after 28 days of treatment to the pre-induction levels [222.1±7.89 – 86.25±6.41].

Table 1: The effect of an aqueous extract of *Imperata cylindrica* roots on the fasting blood glucose in normal, diabetic and diabetic treated rats.

Treatment groups	Mean initial FBS	Mean final FBS
Group A [Non-diabetic Control]	87.25±4.01	90.38±5.62↓
Group B [Diabetic Control]	211.60±2.34	202.00±1.21↓
Group C [Diabetic rats treated with 200mg/kg Aqueous Extract]	204.40±2.61	132.60±4.76↓**
Group D [Diabetic rats treated with 400mg/kg aqueous extract]	212.6±3.93	117.50±7.69↓***
Group E [Diabetic rats treated with 600mg/kg aqueous extract]	217.6±3.27	151.50±10.05↓**
Group F [Positive control diabetic rats treated with 0.5mg/kg Glibenclamide]	222.10±7.89	86.25±6.41↓****

Values are expressed as arithmetic mean ± SEM, N=8. . **P<0.01. ***P<0.001. ****P<0.0001.

The effect of an aqueous extract of *Imperata cylindrica* roots on the Serum testosterone levels in normal, diabetic and diabetic treated rats.

The serum testosterone concentrations exhibited great variations [Table 2]. It was observed that the testosterone level in the non-diabetic group of rats showed slight increase [P<0.05] from 5.62±0.39 to 5.40±0.25. Similarly, the alloxan-induced diabetic untreated group showed a reduction in testosterone level from 3.16±0.14ng/ml to 3.06±0.10 ng/ml [P<0.05]. In contrast, the concentration of the testosterone hormone in groups treated with various doses of *Imperata cylindrica* aqueous extract had statistically significant increases compared pre-treatment levels, [3.35±0.08 to 3.57±10, 3.20±0.17 to 4.40±0.32 and 3.23±0.04 to 4.57±0.15 ng/ml] respectively for the 200, 400 and 600mg/kg body weight of the extract respectively. Glibenclamide 0.5mg/kg showed more spectacular increase in testosterone to levels comparable to the non-diabetic control group [[3.17±0.12 to 5.38±0.27 ng/ml].

Table 2: The effect of an aqueous extract of *Imperata cylindrica* roots on the Serum testosterone [TT] levels in normal, diabetic and diabetic treated rats.

Treatment groups	Mean initial TT [ng/ml]	Mean final TT [ng/ml]
Group A [Non-diabetic Control]	5.61±0.39	5.40±0.25↓*
Group B [Diabetic Control]	3.16±0.14	3.06 ±0.10↓*
Group C [Diabetic rats treated with 200mg/kg Aqueous Extract]	3.35±0.08	3.57±0.10↑****
Group D [Diabetic rats treated with 400mg/kg aqueous extract]	3.20±0.17	4.40±0.32↑****
Group E [Diabetic rats treated with 600mg/kg aqueous extract]	3.23±0.04	4.57±0.15↑****
Group F [Positive control diabetic rats treated with 0.5mg/kg Glibenclamide]	3.17±0.12	5.38±0.27↑****

*P<0.05; **P<0.01; ****P<0.0001.

DISCUSSION

Imperata Cylindrica root is reputed for its potentials in traditional folklore of some parts of Eastern Nigeria for the treatment of impotency and diabetes mellitus among other ailments but no clear scientific evidence is available to back it up. This practice has gained acceptance in these cultural settings but it is yet to be scientifically ascertained. Thus an analysis of the effect of an Imperata cylindrica root aqueous extract on fasting blood sugar and serum testosterone; which are some biochemical parameters of erectile function, in diabetic Wistar rats will go a long way to unravel the physiological basis for its use by some traditional medical practitioners in this clime for the treatment of erectile dysfunction, especially, due to diabetes mellitus..

The type and level of biological activity exhibited by any plant material depends on many factors, including the plant part, geographical source, soil conditions, harvest time, moisture content, drying method, storage conditions, and post-harvest processing. For example, the relatively high temperatures that can be generated

during tissue grinding can denature chemical constituents and the extraction solvent, time period, and temperature can affect the level and composition of secondary metabolites extracted from plant tissues. This was taken into consideration in selecting the extraction methods for this study which also resembles as closely as possible its traditional application [25]. Previous studies have been carried out to find the potential of Imperata cylindrica for medicinal use but none was done using the ones harvested from this environment [17].

Herbal drugs are popular in the treatment of numerous disease conditions [15]. The major merits of herbal medicines seem to be their efficacy, low incidence of side effects, and low cost. An acute and sub chronic toxicity study of the aqueous extract from root of *Imperata cylindrica* in rats showed no abnormality in the test groups as compared with the controls [23]. Based on the established safety of *Imperata cylindrica*, this study investigated a possible scientific basis for its use in Igbo land for the local treatment of erectile dysfunction due to diabetes mellitus by determination of its effect on fasting blood sugar and serum testosterone [28] in a Wistar rat model of Diabetes Mellitus using established protocol. Testosterone deficiency and erectile dysfunction have been shown to occur together especially in Diabetes Mellitus which is a worldwide disorder that affects millions of men and has a negative influence on quality of life [29]. Erectile dysfunction is commonly treated with a prescription of phosphodiesterase 5 [PDE-5] inhibitors [Sildenafil, Tadalafil etc.]. These drugs are potent inhibitors of cyclic guanosine monophosphate [cGMP] -specific PDE-5 and it has been discovered that their enhancement of penile erection in patients with erectile dysfunction involves potentiation of the nitric oxide [NO]-stimulated cGMP signal mediating relaxation of cavernosal smooth muscle during sexual stimulation. They have a high selectivity for PDE5 relative to other PDE isozymes [30]. Studies show that PDE-5 inhibitors work more efficiently when there is a sufficient amount of testosterone present [31]. Several factors might lead to erectile dysfunction but the one due to a deficiency of testosterone in diabetes mellitus can likely be corrected by the use of substances that increase testosterone levels [32].

Alloxan was used to induce diabetes in this study as it is a well- known diabetogenic agent widely used to induce diabetes in animal models [26]. Rats were induced diabetes by the administration of single intraperitoneal dose of Alloxan monohydrate [150 mg/kg]. After 28 days of Alloxan injection; an increase in blood sugar level and decreased testosterone level were observed. These are indices of the negative biochemical effects associated with Alloxan induction of Diabetes Mellitus [33, 34].

Alloxan monohydrate [150 mg/kg, i.p.] led to an elevation of fasting blood glucose levels, which was maintained over a period of 28 days. Four weeks of daily treatment with various doses of *Imperata cylindrica* roots aqueous extract also led to a fall in blood sugar levels for the three analysed doses. The increase in blood glucose level after Alloxan administration may be due to insulin deficiency or resistance state in diabetic rats. Administration of the Aqueous extract of *Imperata cylindrica* root significantly reduced blood glucose level in diabetic rats which represents reversal of insulin resistance or increasing insulin secretion possibly by regeneration of damaged pancreatic β-cells in Alloxan-induced diabetic rats [33].

There was observed variations in the serum testosterone concentrations of the diabetes induced and the diabetic treated Wistar rats. The experimental groups with consumption of minimum, average, and maximum dose of the extract had significant increases compared with control untreated group. The positive control group treated with Glibenclamide also showed statistically significant increase compared to the negative control group [Table 2]. It was observed that the testosterone level in the non-diabetic group of rats showed slight increase [$P<0.05$] from 5.62±0.39 to 5.40±0.25 ng/ml. Similarly, the alloxan-induced diabetic untreated group showed a reduction in testosterone level from 3.16±0.14ng/ml to 3.06±0.10 ng/ml [$P<0.05$]. In contrast, the concentration of the testosterone hormone in groups treated with various doses of *Imperata cylindrica* aqueous extract had statistically significant increases compared to pre-treatment levels, [3.35±0.08 to 3.57±10, 3.20±0.17 to 4.40±0.32 and 3.23±0.04 to 4.57±0.15 ng/ml] respectively for the 200, 400 and 600mg/kg body weight of the extract respectively. Glibenclamide 0.5mg/kg showed more spectacular increase in testosterone to levels comparable to the non-diabetic control group [[3.17±0.12 to 5.38±0.27 ng/ml].

The induction of diabetes mellitus decreased the testosterone level significantly, which was reversed by treatment with aqueous extract of *Imperata cylindrica* root and Glibenclamide. The negative control diabetic group did not show any significant change in testosterone level during the twenty eight days of treatment. The results are indicative of a positive effect of treatment with the extracts of *Imperata cylindrica* roots on testosterone level of diabetic Wistar rats.

Phytochemical analysis of the Imperata cylindrica root extracts show the presence of flavonoids and other potent phytonutrients [24] which had been shown to possess medicinal value [36]. Flavonoids are known to be bioactive antidiabetic principles [37, 38]. Flavonoids are known to regenerate the damaged beta cells in the Alloxan induced diabetic rats and acts as insulin secretagogues [38] and many flavonoids can be effective in reducing free radical damage to cells and other components in body tissue [39]. Flavonoids have also been shown to increase the strength and integrity of the blood vessel walls, lowering risk of blood vessel problems [40]. They have also been found to

be involved in the onset of penile erection and improved sexual performance [41]. *Imperata cylindrica* roots have been used for traditional medicinal purposes in the treatment of numerous disease conditions including Diabetes Mellitus and erectile dysfunction. This research discovered the glucose lowering and testosterone boosting effect of an aqueous extract of *Imperata cylindrica* root and may be proof not only of the antidiabetic properties of *Imperata cylindrica* roots but also of its value in the traditional use for the treatment of erectile dysfunction due to Diabetes Mellitus [42].

CONCLUSION

The administration of an aqueous extract of *Imperata cylindrica* roots to Alloxan induced hyperglycaemic rats demonstrated a reduction in blood sugar level and testosterone levels as compared to Alloxan induced negative control rats. This study demonstrated that an aqueous extract of *Imperata cylindrica* root increased the level of testosterone in testosterone compromised Alloxan treated Wistar rats. Interestingly the increase in the testosterone level was very remarkable and statistically significant in most cases after treatment. This finding may establish the pharmacological basis for the use of extracts of *Imperata cylindrica* root for the Igbo folkloric management of sexual dysfunction especially due to diabetes mellitus.

COMPETING INTERESTS

The authors report no conflicts of interest in this work.

AUTHOR CONTRIBUTION:

Ghasi S.I. and Nwokike M.O.: Designed the study and performed the experiments

Ghasi S.I.: Conducted the data analysis

Nwokike M.O., **Ogbonna and Anusiem C.A.**: Participated in drafting the paper

Nwokike M.O.: Wrote the manuscript. All authors read and approved the manuscript.

ABBREVIATIONS

TRT: Testosterone replacement therapy

HRT: hormone replacement therapy

AICR: Aqueous extract of *Imperata cylindrica* root

UNN: University of Nigeria Nsukka

ELISA: Enzyme-Linked Immunosorbent Assay

FBS: Fasting blood sugar

TT: Serum testosterone

SEM: Standard error of mean

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