



## **Examining the Androgenic Effect of Different Imperata Cylindrica Extracts on Diabetic Wistar Rats**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/EJMP/2021/v32i630396

#### Editor(s):

(1) Dr. Paola Angelini, University of Perugia, Italy.

(2) Prof. Marcello Iriti, Milan State University, Italy.

#### Reviewers:

(1) Indla Ravi, Kerala University Of Health Sciences, India.

(2) Harsh Joshi, India.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/70644>

**Original Research Article**

**Received 20 May 2021**  
**Accepted 27 July 2021**  
**Published 03 August 2021**

### **ABSTRACT**

This was an experimental study that evaluated the effect of aqueous, methanol and chloroform root extracts of Imperata cylindrica on serum levels of testosterone in male Wistar rats. Shade dried Imperata cylindrica roots were pounded and crude extracts prepared using distilled water, methanol and chloroform. Diabetes mellitus was induced with alloxan monohydrate and the diabetic rats were divided into six groups (n=8) and kept in separate cages. Group A rats were Non-Diabetic Rats Treated With Distilled Water. In Group B were placed Alloxan-Induced Diabetic Rats Treated with Distilled Water. Groups C, D and E contained Alloxan-Induced Diabetic Rats Treated with 200 mg/Kg body weight water, chloroform and methanol Imperata cylindrica root extracts in the given order. Group F was the Positive Control with Alloxan-Induced Diabetic Rats Treated with Glibenclamide [0.5 mg/kg body weight]. This treatment was carried out for 28 days sequentially. An analysis of the serum obtained from the rats after 28 days indicate that the

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extracts increased testosterone levels to varying degrees with the methanol extract producing the highest activity of 27.42 percent. This increase is from  $3.23 \pm 0.04$  ng/ml at baseline to  $4.45 \pm 0.11$  ng/ml after 28 days ( $p < 0.0001$ ). The increase due to aqueous extract was equally very statistically significant ( $p < 0.0001$ ) as the baseline concentration of testosterone,  $3.35 \pm 0.08$  ng/ml was increased by 23.70% to  $4.39 \pm 0.32$  ng/ml. The chloroform extract proved to be the least active of the extracts as it only increased the testosterone level by 11.76% from  $3.15 \pm 0.19$  ng/ml at baseline to  $3.57 \pm 0.09$  ng/ml after 28 days ( $p = 0.0213$ ). This was about two and half times lower than the methanol extract that elicited highest activity among the extracts and about four times less than the effect produced by the Group F rats treated with 0.5 mg/kg body weight of glibenclamide. The testosterone levels of Group F rats after 28 days increased to the levels seen in the normal control group that did not receive alloxan, from  $3.17 \pm 0.12$  to  $5.51 \pm 0.25$  ng/ml, a 42.47% change. This result indicates that methanol is the best extractant of the three solvents analyzed.

**Keywords:** Comparative; aqueous; methanol; chloroform; extracts; *imperata cylindrica*; testosterone.

## 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

## 1. INTRODUCTION

Diabetes mellitus is characterized by diverse metabolic disorders including decreased testosterone levels [1] and this informed the use of an animal model of diabetes mellitus to examine three different extracts of *Imperata cylindrica* root for their effect on the testosterone level of Wistar rats. Alloxan monohydrate induces diabetes mellitus in Wistar rats because of its effect on the beta cells of the islets of Langerhans [2,3]. Methods of extraction and the solvent used for extraction are seen to contribute to the potency of plant extracts [4-9]. Aqueous Root extracts of *Imperata cylindrica* had been noted to stimulate increase in testosterone level of diabetic Wistar rats [1,10] and this demanded for an analysis of other extraction solvents to heighten the yield and potency of the extract [11]. Androgen deficiency and erectile dysfunction is widely associated and this is mainly due to a decreased testosterone level [12]. There is a need to correct this form of erectile dysfunction with substances that can stimulate an increase in testosterone level [13]. Different methods of treatment have been used to improve testosterone deficiency including testosterone replacement therapy at a cost of numerous side

effects, thus requiring other potential drug candidates from plants [14]. The grass *Imperata cylindrica* is not only easily available but is mostly seen as a noxious weed [15], and proper utilization will increase the arsenal of plants with potential value as drug candidates for the treatment of testosterone deficiency especially due to diabetes mellitus.

### 1.1 Objectives of the Study

The present study examined the effect of water, methanol and chloroform on the extraction yield of *Imperata cylindrica* root and further investigated the effect of the resulting extracts on testosterone levels. The result of this study points to a solvent to be used for better yield and potency of *Imperata cylindrica* root extract.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

This analysis was reviewed and approved by the University of Nigeria Teaching Hospital-Enugu Health Research Ethics Committee with No. NHREC/05/01/2008B-FWA00002458-1RB00002323 and designed as an in vivo experimental study. The roots of *Imperata cylindrica* was harvested and extracted with chloroform, methanol and water using standard procedures (4 and 17). Alloxan Monohydrate was used to induce diabetes mellitus [16]. The Wistar rats were placed into diabetic and control groups and treatment given with the extracts, glibenclamide and water for a period of twenty eight days in each case [1]. Blood samples were collected for analysis before and after each study. These blood samples were used for the

comparative analyses of the testosterone levels using a standard analytical procedure (17).

## 2.2 Plant Material and Preparation of Extract

The *Imperata cylindrica* roots used for this study were obtained from Awha Imezi in Ezeagu Local Government Area of Enugu State. It was authenticated and identified at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A voucher specimen has been kept in the herbarium with reference number: UNH166. Extraction of *Imperata cylindrica* roots with water, chloroform and methanol solutions was done to resemble as close as possible the way that it is used in traditional medical practice as described previously [1,10,17]. The yields of extracts are shown in Appendix A.

## 2.3 Chemicals and Reagents

Alloxan monohydrate and Glibenclamide were purchased from Bristol chemicals Lagos, Nigeria. The ACCU-CHEK Active glucometer [Roche Diagnostics GmbH], ELISA Kit [Abcam] and other chemicals and reagents (Bristol Chemicals, Nigeria) were of analytical grade.

## 2.4 Animals Grouping and Treatment

Two hundred adult male Wistar rats (150 to 200grams) obtained from the animal house of the University Of Nigeria College Of Medicine, were kept in cages for one week acclimatization with access to standard animal diet and water *ad libitum*. Eighty of these rats were fasted for 10 hours. A single intraperitoneal dose of Alloxan monohydrate, 150 mg/kg body weight was administered as a 5% w/v in distilled water to the rats for the induction of Diabetes mellitus. The induced rats were tested for sufficient levels of hyperglycaemia at 4 weeks post injection after which forty rats with blood glucose levels above 200mg/dl and eight normal rats in which diabetes mellitus were not induced

were selected for treatment with extracts of *Imperata cylindrica*. The forty eight rats were randomly divided into 6 groups of 8 animals each and treated with one of the following: distilled water (10 ml/kg b.w.); aqueous extract of *Imperata cylindrica* roots (200 mg/kg b.w.); methanol extract of *Imperata cylindrica* roots (200 mg/kg b.w.), chloroform extract of *Imperata cylindrica* roots (200 mg/kg b.w.) and glibenclamide (0.5 mg/kg b.w.) for 28 days consecutively (Appendix B). On the 29<sup>th</sup> day the animals were sacrificed and whole blood 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 and serum separated with Pasteur pipettes into sterile sample tubes for the testosterone assay which was carried out using an Enzyme-Linked Immunosorbent Assay [ELISA] Kit [Abcam] [1,10,18].

## 2.5 Statistical Analysis

The obtained values from this study are expressed as mean  $\pm$  standard error of mean [SEM] of all the observations [19,20]. Student t – test was performed to compare the results obtained from each treatment group and p-value of <0.05 was taken as indicating a statistically significant difference [21-23].

## 3. RESULTS

### 3.1 Outcome of Treatment with Water, Methanol and Chloroform Extracts of *Imperata cylindrica*

A study of the yield of water, methanol and chloroform extracts of *Imperata cylindrica* (Appendix A) show that methanol yielded the greatest amount of extract (15.3%) of *Imperata cylindrica* root of the three solvents used (Table 1), followed by the chloroform (12.7%) and water (7.0%).

**Table 1. The result of treatment with water, methanol and chloroform extracts of *Imperata cylindrica***

Solvent	Yield (%)
Water	7.0 %
Methanol	15.3%.
Chloroform	12.7%.

**Table 2. The result of treatment with water, methanol and chloroform extracts of *Imperata cylindrica* root on testosterone levels ng/ml**

Group	Baseline mean± sem	28days mean± sem	% activity change	P-value
A. Normal control	5.62±0.39	5.32±0.24	-5.64	0.5962
B. Diabetic control	3.26±0.13	3.57±0.05	8.68	0.0612
C. Aqueous extract (200mg/kg b.w.)	3.35±0.08	4.39±0.32	23.70	<0.0001
D. Chloroform extract (200mg/kg b.w.)	3.15±0.19	3.57±0.09	11.76	0.0213
E. Methanol extract (200mg/kg b.w.)	3.23±0.04	4.45±0.11	27.42	<0.0001
F. Glibenclamide (0.5 mg/kg body weight)	3.17±0.12	5.51±0.25	42.47	<0.0001

Values are expressed as mean ± SEM, n=8

### 3.2 Outcome of Treatment with Water, Methanol and Chloroform Extracts of *Imperata cylindrica* on Serum Testosterone Levels of Diabetic Wistar Rats

The average testosterone level in the normal untreated rats was 5.62±0.39 ng/ml at the start of the study and remained virtually unchanged at 5.32±0.24 ng/ml twenty eight days later (Table 2). This was a 5.6 percent decrease compared to the baseline value and was statistically insignificant (p=59762). The testosterone levels in the diabetic control group were significantly decreased to 3.26±0.13 ng/ml following induction with alloxan. This value did not witness any significant change over time as the testosterone concentration on the 28<sup>th</sup> day was found to be 3.57±0.09 ng/ml (p=0.0612), representing an 8.68% increase in activity. The three different root extracts of *Imperata cylindrica*, aqueous, chloroform and methanol, increased the testosterone levels of diabetic rats to varying degrees with the methanol extract producing the highest activity of 27.42 percent from 3.23±0.04 ng/ml at baseline to 4.45±0.11 ng/ml after 28 days (p<0.0001). The increase due to aqueous extract was equally very statistically significant (p<0.0001) as the baseline concentration of testosterone, 3.35±0.08 ng/ml was increased by 23.70% to 4.39±0.32 ng/ml. The chloroform extract proved to be the least active of the extracts as it only increased the testosterone level by 11.76% from 3.15±0.19 ng/ml at baseline to 3.57±0.09 ng/ml after 28 days (p=0.0213). This was about two and half times lower than the methanol extract that elicited highest activity among the extracts and about four times less than the effect produced by the

positive control group that were treated with glibenclamide 0.5 mg/kg body weight (p<0.0001). This dose of glibenclamide increased the testosterone level in 28 days to the levels seen in the normal control group that did not receive alloxan, from 3.17±0.12 to 5.51±0.25 ng/ml, a 42.47% change.

### 4. DISCUSSION

The Medicinal and therapeutic effects of several plants which are used in traditional medicine are usually attributed to their methods of extraction [11]. This informed the use of Water, methanol and chloroform for the extraction of *Imperata cylindrica* roots to get the best yield of extract with the hope that this will enhance the potency of the extract [11]. A Wistar rat model for the induction of Diabetes mellitus was used in this study because Diabetes mellitus is characterized by diverse metabolic disorders including decreased testosterone levels [1]. Intra peritoneal injection of Alloxan monohydrate was made as Alloxan is known to cause tremendous damage to pancreatic β-cells leading to the induction of diabetes mellitus in the animals [2,3]. Forty eight rats were selected for the study and placed into six groups of eight rats each [Appendix B]. Treatment with the plant extracts and glibenclamide was administered once in a day for 28 days consecutively. Whole blood was collected by cardiac puncture from each animal into clean dry test tubes 24 h after the last treatment for the measurement of the Testosterone concentrations using a standard procedure [18]. Analysis of the extraction yield of *Imperata cylindrica* using water, methanol and chloroform (Appendix A) show that methanol is the best extractant (15.3%) of *Imperata cylindrica* root of the three solvents used followed

by chloroform (12.7%.) and water (7.0%). Comparison of the effects of different extracts of roots of *Imperata cylindrica* on the testosterone levels of alloxan-induced diabetic rats show variations in the serum testosterone concentrations of the diabetes induced and the diabetic treated Wistar rats. The three different root extracts of *Imperata cylindrica*, aqueous, chloroform and methanol, increased the testosterone levels of diabetic rats with the methanol extract producing the highest activity of 27.42 percent from  $3.23 \pm 0.04$  ng/ml at baseline to  $4.45 \pm 0.11$  ng/ml after 28 days ( $p = 0.0001$ ). The increase due to aqueous extract was equally very statistically significant ( $p = 0.0001$ ) as the baseline concentration of testosterone,  $3.35 \pm 0.08$  ng/ml was increased by 23.70% to  $4.39 \pm 0.32$  ng/ml. The chloroform extract proved to be the least active of the extracts as it only increased the testosterone level by 11.76% from  $3.15 \pm 0.19$  ng/ml at baseline to  $3.57 \pm 0.09$  ng/ml after 28 days ( $p = 0.0213$ ). This was about two and half times lower than the methanol extract that elicited highest activity among the extracts and about four times less than the effect produced by the positive control group that were treated with glibenclamide 0.5 mg/kg body weight ( $p = 0.0001$ ). This dose of glibenclamide increased the testosterone level to the levels seen in the normal control group that did not receive alloxan, from  $3.17 \pm 0.12$  to  $5.51 \pm 0.25$  ng/ml, a 42.47% change in 28 days. 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 general positive effect of treatment with the extracts of *Imperata cylindrica* roots on testosterone level of diabetic Wistar rats. The administration of *Imperata cylindrica* roots extracts to Alloxan induced hyperglycaemic rats demonstrated an increase in testosterone levels as compared with Alloxan induced negative control rats [1,10]. Testosterone is involved in developing male secondary sex characteristics such as the growth of body hair and facial hair, the deepening of the voice, and increased penis and testes size, sperm production and sex drive [24]. Low testosterone levels in male subjects is associated with diminished sexual function, erectile dysfunction, reduced sperm count, loss of muscle mass or hair and reduced sex drive [25]. Testosterone can be synthesised and Testosterone Replacement Therapy (TRT) is often used to supplement the natural testosterone production [26], but this is generally only recommended as a short-term treatment because it comes with a high risk of side effects

[27-29]. Plant extracts that boost testosterone synthesis are a plus to pharmacotherapy as potential candidates for the treatment of sexual dysfunction in males. This research discovered that the solvent used for extraction is significant for the effect of the extract on diabetic Wistar rats. It demonstrated that methanol extract of *Imperata cylindrica* root increased the level of testosterone in testosterone compromised Alloxan treated Wistar rats better than the aqueous and chloroform extracts.

## 5. CONCLUSION

The results of this study show that the used solvents took an important role in the yield of extracts and the tested biological activity. Methanol was identified as the most effective solvent for the extraction, resulting in the highest extraction yield (15.3%) followed by chloroform (12.7%) and water (7.0%). Therefore, methanol is recommended as the optimal solvent to obtain high content of *Imperata cylindrica* extract. Though chloroform produced a greater yield of extract (12.7%.) than water (7.0%), the effect of the aqueous extract on testosterone level at a dose of 200mg/kg body weight was greater than that of the chloroform extract. Further phytochemical analysis will compare the yield of these extracts to identify the active phytonutrients with effect on testosterone levels of diabetic Wistar rats.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

This analysis was reviewed and approved by the University of Nigeria Teaching Hospital-Enugu Health Research Ethics Committee with No. NHREC/05/01/2008B-FWA00002458-

1RB00002323 and designed as an in vivo experimental study.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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