

Evaluation of Some Reproductive Properties of *Hibiscus sabdariffa* in Alloxan - Induced Diabetic Male Wistar Rats

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Abstract: *Diabetes Mellitus is a disease condition characterized by persistent hyperglycaemia. It is fast becoming a public health menace due to rapidly increasing incidence of the disease on a global scale. The present study was aimed at evaluating effects of aqueous calyx extract of Hibiscus sabdariffa on reproductive functions of alloxan - induced diabetic male Wistar rats. The animals were assigned into four (4) groups of five (5) rats each. Group 1 was non diabetic (negative control). Group 2 was diabetic control that was not treated. Group 3 was diabetic and treated with 250mg/kg of Hibiscus sabdariffa (calyx) extract while group 4 was diabetic and treated with 500mg/kg of Hibiscus sabdariffa (calyx) extract. Induction of diabetes was done with 150mg/kg of alloxan injected intraperitoneally. Extract administration which was by oral gavage lasted for a period of four (4) weeks. At the end, animals were sacrificed and blood samples were collected for hormonal assays. The epididymis was lacerated to extract sperm cells for sperm analysis. Results revealed that the extract significantly ($P < 0.05$) increased serum testosterone level and percentages of actively motile sperm cells and sperm cells with normal morphology in diabetic male Wistar rats. It was concluded that, aqueous calyx extract of Hibiscus sabdariffa ameliorated diabetes induced decline in some reproductive functions of male Wistar rats.*

Keywords: *Hibiscus sabdariffa*, Wistar rats, Reproductive functions, Diabetes Mellitus

1. Introduction

Diabetes mellitus is a chronic, fatiguing and expensive disease arising from persistent hyperglycemia. Persistent hyperglycemia destroys almost all cell types in the body [1]. Diabetes mellitus is rapidly becoming a major public health problem and requires special attention and actions towards its control [2].

There has also been confirmed reports indicating that diabetes possess harmful effects on both male and female reproductive functions [3] [4], and its effects can be noted in increasing cases of infertility amongst diabetics [5]. A large proportion of diabetics encounter disorder in sexual function, comprising a reduction in libido and increasing impotence [6]. In addition, diabetic men are susceptible to various sexual difficulties which is worsened by progressive physical diseases and damaging psychological posture [7].

In a report, there were 382 million (or 8.3% of adult population) people worldwide who suffered from diabetes by the end of 2013 [8]; of which 80% live in low - and - middle - income countries as reported by International Diabetes Federation (IDF). This figure is extrapolated to get to 592 million in sub - Saharan Africa. Nigeria possess significantly high number of people with diabetes with an extrapolated 3.9 million people (or an extrapolated prevalence of 4.99%) of the adult population aged 20–79 -

year - old [8]; Even so, there were still about 1.8 million Nigerians with unknown diabetic status in 2013 [9].

The management of diabetes has posed significant challenge to both diabetics and health care providers. In orthodox medical practice, the present therapies used in the treatment of diabetes mellitus include the glucose - lowering drugs such as, insulin secretagogues (sulfonyl - ureas, meglitinides), insulin sensitizers (biguanides, metformin, thiazolidine - diones), α - glucosidase inhibitors (miglitol, acarbose) etc. These agents have been associated with reduced efficiency and occasionally give rise to harmful side effects such as liver injury, weight gain, hypoglycemia, cardiopathy, and bloating [10] [11]. As a result, the search for new anti - diabetic drugs with less side effects, which may also improve reproductive functions in diabetics has become an important area in drug research. To this end, various medicinal plants are being investigated for any possible anti - diabetic and reproduction enhancing effects. *Hibiscus sabdariffa* is a medicinal plant which is popular in Asian and African countries including Nigeria where its calyx is used in the production of both hot and cold beverages consumed as refreshment. It is also considered to have various medicinal properties. The aim of the present study therefore, is to evaluate some reproductive properties of aqueous calyx extract of *Hibiscus sabdariffa* in alloxan - induced diabetic male Wistar rats.

2. Materials & Methods

2.1 Plant Material/ Identification/ Extraction

Dried mature red calyces of *Hibiscus sabdariffa* were purchased from Rumuokoro market in Port Harcourt, Rivers state, Nigeria. It was identified and authenticated by a taxonomist at the herbarium unit of Plant Science and Biotechnology Department, University of Port Harcourt, Nigeria, with Herbarium number, UPH/P/254.

2.2 Preparation of *Hibiscus sabdariffa* Aqueous Calyx Extract

The extraction was carried out using the modified method of Pi - Jen and Brain [12]. Using a weighing balance, two kilogram of *Hibiscus sabdariffa* calyx was weighed, which was then grounded to powder. After grinding, it was dissolved in 2 litres of distilled water which was measured with a measuring cylinder for complete 24 hours, during which it was well macerated i. e shaken properly in a maceration jar. A glass funnel and white handkerchief were used for filtration. The handkerchief was spread over the funnel and the funnel was placed in 1000ml beaker, the filtrate was then carefully poured into the white - handkerchief which was carefully filtered through the funnel into the beaker. In order to obtain a clear filtrate what - man filter paper was used for a second filtration.

After observing a clear filtrate, the filtrate was then poured into an evaporating dish and placed on a water bath at a temperature of 40 - 50 degree Celsius where it gradually evaporated leaving the extract in a paste form. The crude extract which was stored in the refrigerator for use [12] [13]; was later reconstituted into 250mg and 500mg/kg body weight and used for animal oral experiments. The lethal dose (LD50) of *Hibiscus sabdariffa* calyx extract in rats was discovered to be above 5000mg/kg body weight [14].

2.2 Animal Models

Adult male Wistar rats reared in the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt were used for this study. They were chosen at random, placed in neat cages and allowed two weeks to acclimatize. They were nurtured under favourable conditions involving exposure to 12 - hour light and 12 - hour dark schedules, with surrounding environmental temperature of 25°C±2°C and relative humidity of 55 - 65%. They had access to water and feeds *ad - libitum*. Animal handling was done with optimum care and their cages were cleaned and beddings were changed on daily basis. The experiment conformed with standard institutional guidelines [15].

2.3 Experimental Design

Twenty (20) male Wistar rats weighing between 120 - 170g were randomly divided into four (4) groups of five animals each described as follows -:

- Group 1: Non diabetic (Negative control)
- Group 2: Diabetic untreated (Positive control)
- Group 3: Diabetic treated with 250mg/kg bw of *Hibiscus sabdariffa* aqueous calyx extract

- Group 4: Diabetic treated with 500mg/kg bw of *Hibiscus sabdariffa* aqueous calyx extract

The entire treatment period lasted for a period of four (4) weeks and administration was done once daily through oral gavage.

The sample size adopted in this study was by the use of crude method based on law of diminishing return [16].

2.4 Induction of Diabetes

Alloxan was intraperitoneally injected at a single dose of 150mg/kg body weight in overnight fasted rats. Fasting blood glucose levels were measured after three days and the rats with blood glucose level above 200 mg/dl were considered diabetic.

2.5 Sperm Analysis

The epididymis was lacerated to extract the sperm and then mixed with 0.5% Eosin. They were evaluated using the objective lens. 10 - 12 fields were evaluated to check for viable cell which is the percentage of stained cells as against the un - stained ones. Also 10 - 12 fields were evaluated to check for the sperm parameters. The sperm count was determined using counting chamber. 1: 20 dilution of the semen was done using normal saline. The chamber was filled with the diluents. The 4 x 16 squares were counted and multiplied by 100, for example, number of cells counted x 100, 000 = sperm count [17].

2.6. Hormonal Assay

Testosterone, follicle stimulating hormone and luteinizing hormone assay were done using standard methods as described by Tiets in 1986 [18] for testosterone and recorded in ng/ml and Layman in 1997 [19], for follicle stimulating hormone and luteinizing hormone and recorded in m/μ/ml.

2.7 Statistical Analysis

This was accomplished by the application of SPSS version 23. One - way ANOVA was utilized to analyze and compare the mean of the various groups. The mean and standard errors of mean were calculated and the experimental groups compared to control groups. Values of $p < 0.05$ was considered significant. Results were expressed as mean ± standard error of mean (SEM)

3. Results

3.1 Presentation of Result

Table 1: Effect of extract of *Hibiscus sabdariffa* on serum hormone levels of rats

Groups	FSH (m/μ/ml)	LH (m/μ/ml)	T (ng/ml)
Negative Control	0.48±0.03	1.05±0.04	2.24±0.28
Diabetic Control	0.37±0.05	1.11±0.05	1.41±0.11
250mg/kg	0.48±0.06	1.17±0.08	4.05±0.45 ^{*#}
500mg/kg	0.50±0.04	1.18±0.08	3.38±0.60 ^{*#}

Values expressed as mean±SEM. n=5. *, # Significantly different when compared to negative and diabetic controls respectively (P<0.05).

Table 2: Effect of extracts of *Hibiscus sabdariffa* on sperm parameters of rats

Groups	Viable Cells (%)	Normal Morphology (%)	Actively motile cells (%)	Sperm count (x 10 ⁶ /ml)
Negative Control	81.00±3.32	82.00±2.55	76.00±4.30	146.00±17.78
Diabetic Control	73.00±6.82	64.00±5.34*	61.00±6.40*	106.00±30.59
200mg/kg (Extract)	81.00±3.31	78.00±2.00#	76.00±4.30#	150.00±7.07
400mg/kg (Extract)	80.00±3.54	82.00±3.39#	82.00±3.39#	134.00±17.61

Values expressed as mean±SEM. n=5. *, # Significantly different when compared to negative and diabetic controls respectively (P<0.05).

Table 3: Effect of extracts of *Hibiscus sabdariffa* on testicular weight of rats

Groups	Right testis (g)	Left Testis (g)
Negative Control	1.00±0.04	0.96±0.04
Diabetic Control	0.94±0.17	1.00±0.14
200mg/kg (Extract)	1.08±0.21	1.06±0.19
400mg/kg (Extract)	0.80±0.16	0.96±0.19

Values expressed as mean ± SEM. n=5.

4. Discussion

This research study evaluated some reproductive properties of aqueous calyx extract of *Hibiscus Sabdariffa* in alloxan - induced diabetic male Wistar rats. Following four (4) weeks of oral administration of the extract on diabetic male rats, the result revealed that follicle stimulating hormone and luteinizing hormone were not significantly (P< 0.05) altered by the effects of induced diabetes lasting four (4) weeks, since the serum concentration of the hormones did not change significantly in the diabetic control group. However, the serum concentration of testosterone was significantly (P< 0.05) reduced in the diabetic control group suggesting that diabetes may have caused changes that led to a reduction in production of testosterone hormone. But this situation improved in the extract administered groups as the concentration of testosterone in both groups of the extract (250mg/kg and 500mg/kg bw) was significantly increased when compared to the untreated non diabetic and diabetic groups respectively. Although, this increase in testosterone was not dose dependent; this observation is in agreement with the findings of Ezike *et al*, [20] who reported an increase in testosterone level of rats treated with *Hibiscus sabdariffa* extract. This increase could be due to the effects of flavonoids which were discovered to be bioactive component of the extract in this study. Flavonoids were reported to cause an increase in testosterone level in rats by Akpantah *et al.*, [21]. But this finding does not support the findings in a study [22] which reported an improvement in follicle stimulating hormone but no improvement in testosterone and luteinizing hormone levels in streptozotocin induced diabetic Wistar rats administered with aqueous *Hibiscus sabdariffa* calyx extract. Furthermore, Nnabufo and

Olusanya [23] reported a significant decrease (P<0.05) in circulating levels of testosterone, follicle stimulating hormone and luteinizing hormone in rats when orally administered with 250 mg/kg of aqueous *Hibiscus sabdariffa* calyx extract for 28 days. The differences in findings may be due to the methods used in induction of diabetes and different methods of extraction.

The result of the present study also reveals that the percentage of sperm cells with normal morphology and percentage of actively motile cells were significantly (P< 0.05) decreased in the untreated diabetic rats. However, there was a significant increase in these parameters in the rats that received extracts at different doses. The ability of the extract to improve these two parameters of sperm quality could be due to its ability to increase testosterone level. This finding is consistent with those in a report [24] which stated that defected sperms were significantly lowered in mice treated with the extract and another [22] which stated that streptozotocin - induced diabetic rats administered with aqueous *Hibiscus sabdariffa* calyx extract showed improvement in sperm quality. However, the findings in this study does not support the reports of a study [25] which stated that significant (p<0.05) decrease in sperm counts, sperm motility and viability occurred in animals treated with *Hibiscus sabdariffa* extract compared with the control group and the findings of Yomna [26] who stated that the impacts of various doses of *Hibiscus sabdariffa*, either cold or boiled, alter normal sperm morphology and testicular ultrastructure and adversely influence the male reproductive fertility in albino mice. The difference in findings may be due to different methods of extraction and the doses of extract used in the various studies.

The observation from this study may be explained from the fact that increase in testosterone could cause an improvement on the sperm morphology and percentage of actively motile cells in the extract treated animals when compared to the untreated ones.

Boeri *et al.*, [27] in a recent study involving men attending a fertility clinic also found that men suspected of pre - diabetes had more damage to sperm DNA and were more likely to have unexplained azoospermia (no sperm in the ejaculate) and had lower testosterone levels and more disruption of other fertility hormones, compared to men attending the clinic without clinical signs of pre - diabetes. In addition, abnormal glucose homeostasis adversely affected male reproductive functions in the male gamete while testicular function and spermatogenesis are affected in both type 1 and type 2 diabetic men; nonetheless, the current study revealed that the calyx extract of *Hibiscus Sadariffa* was able to reverse the effects of diabetes on these reproductive parameters. The testicular weight was not significantly affected in this study. The bioactive compounds in the calyx extract of *Hibiscus sabdariffa*, such as flavonoids may be responsible for the observed actions of the extract as flavonoids significantly elevates serum level of the male sex hormone.

5. Conclusion

This study has shown that testosterone hormone level, percentages of sperm cells with normal morphology and actively motile cells were significantly reduced in diabetic male Wistar rats. However, the aqueous calyx extract of *Hibiscus sabdariffa* significantly improved these parameters in diabetic Wistar rats.

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