The Journal of Anatomical Sciences
Email: journalofanatomicalsciences@gmail.com

J. Anat Sci 15(1)

The Effect of Aqueous Extract of Calyx of *Hibiscus sabdariffa* on the Testis of Alloxan-induced Diabetic Adult Wistar Rat

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ABSTRACT

Diabetes mellitus contributes to male sexual dysfunction and infertility by causing oxidative damage to the testis. Over the years, the use of herbs like *Hibiscus sabdariffa* for the treatment of ailments has garnered worldwide recognition. However, its impact on male infertility due to Type1 diabetes is not clear, hence, this study. Twenty adult male Wistar rats were divided into four groups of five animals each. Group A was given normal rat feed and water as the Control; Group B was administered 120 mg/kg body weight of Alloxan Monohydrate, Group C was administered 200 mg/kg body weight of *Hibiscus sabdariffa* leaf extract, Group D was administered 120 mg/kg body weight of Alloxan monohydrate + 200 mg/kg body weight of *Hibiscus sabdariffa* leaf extract. All the treatments were given daily orally. Twenty-four hours after the last administration, the animals were anesthetized under chloroform and dissected. The testicular tissues were harvested, weighed and fixed in 10% formal saline for histological studies. Sperm cells were obtained from the epididymis for analysis of sperm quality. The analysis of sperm morphology, sperm motility and sperm count showed better outcome in treatment with *Hibiscus sabdariffa* compared with diabetes-induced rats. Rats treated with *Hibiscus sabdariffa* following diabetes induction revealed fewer lesions and damage of the testicular tissue compared to the diabetic rats. The findings of this study suggest that the aqueous leaf extract of *Hibiscus sabdariffa* has the potential to help in the management of male infertility due to Type 1 Diabetes Mellitus.

Keyword: Type 1 diabetes mellitus, *Hibiscus sabdariffa*, alloxan monohydrate

INTRODUCTION

Diabetes Mellitus is a chronic progressive disease mainly featured by high blood glucose level[1]. It is a common metabolic disease and affects millions of patients worldwide and its prevalence is rapidly rising in developing countries[1.] This disease originates from the failure of insulin secretion due to Beta cell destruction in the pancreas or the insulin resistance of the peripheral tissues due to prolonged over-exposure of insulin [2]. Alloxan, which is chemically known as 5, 5- dihydroxyl pyrimidine-2, 4, 6-trione is a derivative of urea and a common diabetogenic compound[3]. Alloxan induces diabetes by a mechanism which basically involves partial degradation of the Beta cells of the pancreatic islets which gives rise to subsequent compromise in the quality and quantity of insulin produced[4]. Persistent hyperglycemic condition may cause overproduction of free radicals by activating polyol and glucose pentose pathway, increasing glucose auto oxidation and lipid peroxidation as well as disturbance of the antioxidant defense system. The resultant free radicals bring about intracellular oxidative stress. Tissue damage exerted by activity of free radicals is an important factor in the pathogenesis of Diabetes Mellitus complication[5]. The calyx of *Hibiscus sabdariffa* is widely used in medicinal applications and its benefits have been reported in antioxidant and hypoglycemic activities[6]. Few studies have been conducted on the effect of *Hibiscus sabdariffa* on the male reproductive system and they have produced conflicting results. However, scarce evidence is available on the role of *Hibiscus sabdariffa* in preventing testicular damage due to Type-1 diabetes. This study was aimed at investigating the effect of aqueous extract of calyx of *Hibiscus sabdariffa* on the testis of Alloxan-induced diabetic Wistar rat.

MATERIALS AND METHODS

This research was carried out in the Department of Anatomy, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. Ethical approval was obtained from Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC).

Substance of Study

The dried calyces of *Hibiscus sabdariffa* (zobo leaves) used were procured from the Nkwo market of Nnewi, Anambra State. The plant was authenticated at the Herbarium Unit, Botany Department, Nnamdi Azikiwe University. The Alloxan Monohydrate used was produced by Titan Biotech Ltd.

Experimental Animals

A total of 20 Adult male Wistar rats were obtained from the Department of Physiology, Nnamdi Azikiwe University, Nnewi Campus and housed in the Central Animal House, Nnamdi Azikiwe University College of Health Sciences, Nnewi Campus. The rats' weights ranged from 105 - 144 g. The rats were kept in Perspex cages at optimum temperature, 12hrs light/dark cycle and fed with commercial grower mesh and water *ad libitum*. The animals were fed with poultry feed known as Top Feed which was manufactured by Premier feed mills company limited (a subsidiary of Flour Mills Nigeria plc) in Sapele, Delta State and purchased from Nkwo Market, Nnewi Anambra State. The rats were properly handled with the use of hand gloves. Each of the rats was identified using non-invasive method, that is; the use of permanent markers of different colors. The experiment was carried out in accordance with current rules and guidelines established for care of laboratory animals. The rats were acclimatized for 2 weeks before administration commenced.

Experimental Design

The rats were randomly grouped as follows:

Group A: control; received normal feed and water daily throughout the period of experiment; Group B: received 120 mg/kg of Alloxan monohydrate; Group C: received 200 mg/kg of *Hibiscus sabdariffa* leaf extract; Group D: received 120 mg/kg of Alloxan monohydrate + 200 mg/kg of *Hibiscus sabdariffa* leaf extract.

At the end of the experiment, 2 rats in each group were sacrificed by cervical dislocation and the semen quality was analyzed. The testes of each rat were weighed and immediately fixed in a labeled container specific for each rat.

Histological Evaluation Sample Collection

Tissue samples were collected from all groups and fixed using Bouin's fluid. The tissues were then dehydrated by immersing in 70%, 90%, 95% and absolute alcohol. The tissues were further passed through Xylene for clearing. The tissues were embedded in paraffin wax and sectioned using a rotary microtome. The Hematoxylin and Eosin (H& E) stains were used and the tissues slides were examined.

Data Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 25. Data obtained (sperm motility, sperm morphology, total sperm count, relative testicular weight and blood glucose level) were analyzed using ANOVA followed by Post-Hoc LSD multiple comparison. Body weight was analyzed using a T-test and values were considered significant at $p<0.05$.

RESULTS

Findings revealed a significant increase ($p<0.05$) in the body weight in-Group A, while Groups C and D had a non-significant increase ($p>0.05$); Group B had a non-significant ($p>0.05$) decrease in the bodyweight when the initial weight was compared to the final weight (Table 1).

Also demonstrated was a significant ($p<0.05$) increase in the relative testicular weight in-Group A compared to B, Group C had a non-significant ($p>0.05$) decrease, and D had a non-significant ($p>0.05$) increase compared to group B (Table 2).

The results of sperm parameters indicated a significant ($p<0.05$) decrease in the normal sperm cells in group B compared to group A, groups C and D had significant increase compared to group B. The abnormal sperm cells result showed a significant ($p<0.05$) increase in-group B compared to group A, groups C and D had significant decrease compared to group B (Table 3). There was a significant ($p<0.05$) decrease in the active motility in-Group B compared to A; Group C had a non-significant ($p>0.05$) increase and D had significant ($p<0.05$) increase compared to Group B. However, the non-motile sperm showed a significant ($p<0.05$) increase in the active motility in-Group B compared to A, Groups C had a non-significant ($p>0.05$) decrease and D had significant ($p<0.05$) decrease compared to Group B. The total sperm count result showed significant decrease ($p<0.05$) in Group B compared to A; Groups C and D had significant ($p<0.05$) increase compared to Group B (Table 4).

The findings of blood glucose level revealed a significant ($p<0.05$) increase in the blood glucose level in groups B and D, while group C had a non-significant ($p>0.05$) increase compared to group A at day 0. At day 7, 14, and 21, a significant ($p<0.05$) increase in the blood glucose was observed in-group B compared to group A, while groups C and D had a significant ($p<0.05$) decrease compared to group B (Table 5).

Table 1: Effect of aqueous extract of *Hibiscus sabdariffa* on body weight following Alloxan-induced toxicity

		Mean±SEM	**BWD (g)**	**p-value**	**t-value**
Group A (Positive control)	Initial weight (g)	109.75±2.05	42.75	0.02[a]	-4.06
	Final weight (g)	152.50±9.24			
Group B (Diabetic control)	Initial weight (g)	182.33±28.34	-45.00	0.37[b]	-1.14
	Final weight (g)	137.33±10.91			
Group C (200mg/kg of A*HS*)	Initial weight (g)	125.33±6.88	65.00	0.09[b]	-3.14
	Final weight (g)	190.33±14.49			
Group D (DM + 200mg/kg of A*HS*)	Initial weight (g)	123.00±3.39	39.50	0.18[b]	-1.73
	Final weight (g)	162.50±22.35			

AHS: Aqueous extract of *Hibiscus sabdariffa*, SEM: Standard error of mean, BWD: Body weight difference, [a] (significant), [b] (not significant).

Table 2: Effect of aqueous extract of *Hibiscus sabdariffa* on relative testicular weight following Alloxan-induced toxicity

		MEAN ±SEM	p-value	f-value
Relative testicular weight (g)	Group A (Positive control)	0.51 ±0.12	0.02 [a]	
	Group B (Diabetic control)	0.93 ±0.15		3.83
	Group C (200 mg/kg of A*HS*)	0.84 ±0.06	0.56 [b]	
	Group D (DM + 200 mg/kg of A*HS*)	0.97 ±0.01	0.43 [b]	

AHS: Aqueous extract of *Hibiscus sabdariffa*, SEM: Standard error of mean, [a] (significant), [b] (not significant).

Table 3: Effect of aqueous extract of *Hibiscus sabdariffa* on sperm morphology following Alloxan-induced toxicity

		MEAN±SEM	p-value	f-value
Normal sperm cells (%)	Group A (Positive control)	81.67±4.40	0.00 [a]	
	Group B (Diabetic control)	55.00±2.88		8.53
	Group C (200 mg/kg of A*HS*)	81.66±1.66	0.00 [a]	
	Group D (DM + 200 mg/kg of A*HS*)	81.66±7.26	0.03 [a]	
Abnormal sperm cells (%)	Group A (Positive control)	18.33±.40	0.01 [a]	
	Group B (Diabetic control)	41.66±.66		
	Group C (200 mg/kg of A*HS*)	18.33±1.66	0.01 [a]	7.00
	Group D (DM + 200 mg/kg of A*HS*)	18.33±7.26	0.01 [a]	

[a] (significant)

Table 4: Effect of aqueous extract of *Hibiscus sabdariffa* on sperm motility and total sperm count following Alloxan-induced toxicity

		MEAN ±SEM	p-value	f-value
Active motility (%)	Group A (Positive control)	83.33±1.67	0.02[a]	
	Group B (Diabetic control)	40.00±5.77		3.49
	Group C (200 mg/kg of A*HS*)	53.33±21.85	0.43[b]	
	Group D (DM + 200 mg/kg of A*HS*)	81.67±3.33	0.03[a]	
Non-motile (%)	Group A (Positive control)	16.67±1.67	0.01[a]	
	Group B (Diabetic control)	60.00±5.77		3.49
	Group C (200 mg/kg of A*HS)*	46.67±21.85	0.43[b]	
	Group D (DM + 200 mg/kg of A*HS*)	18.33±3.33	0.03[a]	
Total sperm count (x10^6/mls)	Group A (Positive control)	636.67±37.12	0.00[a]	
	Group B (Diabetic control)	396.67±37.12		11.87
	Group C (200 mg/kg of A*HS*)	553.33±17.63	0.01[a]	
	Group D (DM + 200 mg/kg of A*HS*)	636.67±35.28	0.00[a]	

[a] (significant), [b] (not significant).

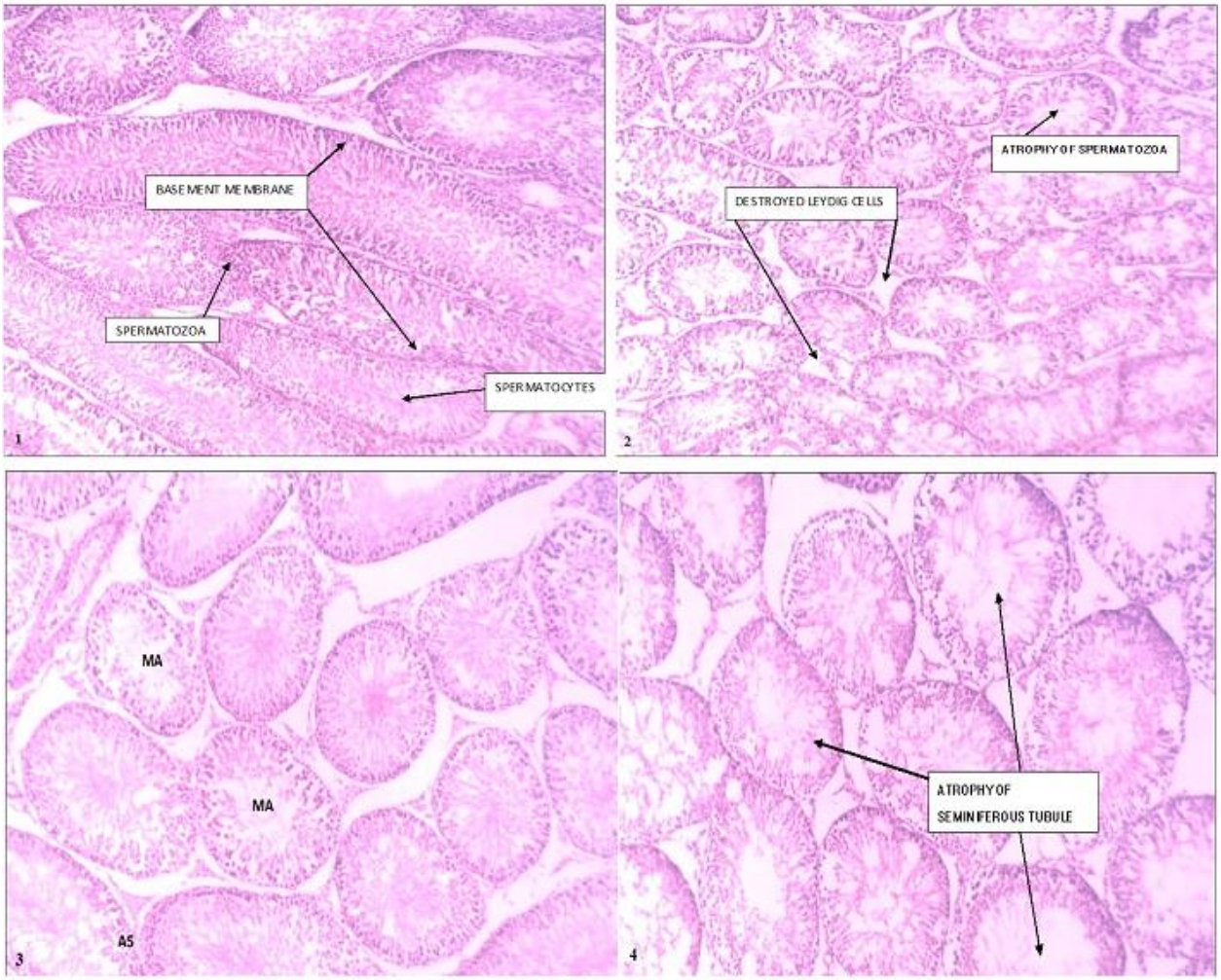
Table 5: Effect of aqueous extract of *Hibiscus sabdariffa* on blood glucose level following Alloxan-induced toxicity

		MEAN±SEM	**p-value**	**f-value**
Day 0 (mg/dl)	Group A (Positive control)	80.00±1.78		
	Group B (Diabetic control)	461.80±63.78	0.00[a]	25.15
	Group C (200 mg/kg of A*HS*)	86.50±4.17	0.92[b]	
	Group D (DM + 200 mg/kg of A*HS*)	474.00±45.01	0.00[a]	
Day 7 (mg/dl)	Group A (Positive control)	80.00±1.95	0.00[a]	
	Group B (Diabetic control)	422.50±34.24		69.95
	Group C (200 mg/kg of A*HS*)	80.25±0.75	0.00[a]	
	Group D (DM + 200 mg/kg of A*HS*)	243.75±18.63	0.00[a]	
Day 14 (mg/dl)	Group A (Positive control)	82.75±.65	0.00[a]	
	Group B (Diabetic control)	456.66±23.33		293.71
	Group C (200 mg/kg of A*HS*)	79.33±1.45	0.00[a]	
	Group D (DM + 200 mg/kg of A*HS*)	119.75±3.79	0.00[a]	
Day 21 (mg/dl)	Group A (Positive control)	80.00±2.04	0.00[a]	
	Group B (Diabetic control)	480.67±17.90		567.18
	Group C (200 mg/kg of A*HS*)	76.33±4.25	0.00	
	Group D (DM + 200 mg/kg of A*HS*)	90.75±3.06	0.00[a]	

AHS: Aqueous extract of *Hibiscus sabdariffa*, SEM: Standard error of mean, BWD: Body weight difference, [a] (significant), [b] (not significant). $p<0.05$

Histological Findings

Findings revealed that the testicular tissue of Group B induced with diabetes presented with intense damage to the tissue architecture. Leydig cells were destroyed and there was atrophy of spermatozoa. In Group C, there was mild atrophy of the seminiferous tubules but normal active sperm cells remain present. In Group D, the atrophy present was reduced compared to that of the diabetic group, Group B.



Figures 1, 2, 3 & 4: Showing testicular tissues (1) with spermatocytes, basement membrane, and spermatozoa; (2) showed testis with destroyed Leydig cells and atrophy of spermatozoa; (3) showed testis appearing with active spermatogonia (**AS**) and mild atrophy (**MA**); (4) showed testis with atrophy of seminiferous tubule (H&E X 100).

DISCUSSION

The results of this study shows that there was a significant increase in the body weight of animals in the control group. This could be physiological as they were only exposed to water and feed. The animals in the experimental groups had a statistically similar results when the initial and final body weights were compared. The relative testicular weight showed differences in the various groups. There was a significant decrease in the relative testicular weight of experimental animals that received Alloxan monohydrate compared to that of control group. This could be due to the oxidative damage brought about by Diabetes. This supports the claim of Barkabi-Zanjani[5], which states that hyperglycemia can reduce the weight and mass of testicular tissue in diabetic rodents. The experimental group that received *H. sabdariffa* had a non-significant decrease compared to the control group and the experimental group that received both Alloxan monohydrate and *H. sabdariffa* had a non-significant increase in the relative testicular weight compared to the group that received only Alloxan monohydrate (induced with diabetes). This could be due to the antioxidant properties of *H. sabdariffa*. It was also observed that administration of aqueous extract of *H. sabdariffa* on rats with diabetes

resulted in a slight increase in the organ weight. This suggests the potential ability to correct shrinkage of the testis as is common in diabetic conditions. If the dose is adjusted, it is possible to produce better results.

The animals that received both Alloxan monohydrate and *H. sabdariffa* presented a general improvement in results of sperm parameters compared to the diabetic group. This shows the ability of *H. sabdariffa* to improve sperm quality in diabetic conditions. This is in line with a study conducted by Idris[8] which was designed to investigate the effects of *H. sabdariffa* UKMR-2 variety on sperm functioning of Streptozotocin-induced diabetic rats. Male Sprague-Dawley rats were used and it was documented that the quality of sperm in the group induced with diabetes and administered *H. sabdariffa* extract was improved with significantly higher sperm concentrations and sperm motility as well as lower percentage of sperm abnormality as compared to the diabetic group.

The results for blood glucose level revealed that there was a significant increase in the blood glucose level in the experimental group induced with diabetes and the group to which Alloxan monohydrate and the *H. sabdariffa* extract were administered, while the group that received the *H. sabdariffa* extract had a non-significant increase compared to the control group at day 0. At days 7,14 and 21, a significant increase in the blood glucose level was noticed in the experimental group induced with diabetes compared to the control group. The experimental groups that received the H. sabdariffa extract and a combination of the Alloxan monohydrate and H. sabdariffa extract had a significant decrease in blood glucose level compared to the group that received only Alloxan monohydrate. This result indicates that *Hibiscus sabdariffa* is potent in reducing blood glucose level. This is similar to the findings of Ojulari *et al.*,[9] where it was documented that *Hibiscus sabdariffa* may have significant effects on blood glucose level in a dose dependent fashion. The improvement of blood glucose level might be due to the protective effect exerted by the antioxidant properties contained in *Hibiscus sabdariffa* which results in prevention of further destruction of Beta cells[2].

The histopathological findings of this study revealed that the testicular tissue of Group induced with diabetes presented with intense damage to the tissue architecture. Leydig cells were destroyed and there was atrophy of spermatozoa. This is as a result of tissue damage caused by the activity of free radicals in diabetic states. Budin[2] opined that prolonged and constant exposure to free radicals in the diabetic state leads to destruction of testicular tissue. The testicular tissue is highly vulnerable to oxidative damage which is contributed by the abundance of polyunsaturated fatty acid content in its structure. The findings of Barsiah[10] showed similar results where the test group induced with Type 1 diabetes mellitus showed significant reduction in testis weight, diameter, the number of cells in the seminiferous tubule, the layer thickness of the germ cell from the basal membrane to the lumen of tubules, and also seminiferous tubule thickness. In the rats that received *H. sabdariffa* extract, there was mild atrophy of the seminiferous tubules but normal active sperm cells remain present. This effect can be attributed to anti-steroidogenic properties of one or more constituents of *Hibiscus Sabdariffa*. In the case of rats that received both Alloxan monohydrate and *H. sabdariffa*, the atrophy present was reduced compared to that of the diabetic group. This points to the restorative properties of *Hibiscus sabdariffa* extract on testicular tissue damaged by Type 1 diabetes mellitus. The restorative and protective effects of this extract may be due to its ability to clean up free radicals which in turn results in a reduction in the oxidative damage of the testis. In a research carried out by Budin[2], it was documented that in the group induced with diabetes and administered *Hibiscus sabdariffa* Polyphenol-rich extracts, the appearance of all tubules were normal and all the spermatogenic cells were normally visible. This disparity in results may be due to the fact that in this present study, aqueous extract of the calyx of *Hibiscus sabdariffa* was used and not polyphenol-rich extract. The different breeds of experimental animals used and the different agents used to induce Type 1 diabetes mellitus may also contribute to the slight disparity in results.

The findings of this study imply caution in the usage of *Hibiscus sabdariffa* as toxicological examinations showed the relative safety of *Hibiscus sabdariffa* and the potential adverse effects it can have on sperm motility and sperm count. In cases of Type 1 Diabetes Mellitus, it was seen to improve the general situation but intake of *Hibiscus sabdariffa* without a predisposed illness may result in mild alterations of testicular tissue, a decrease in active sperm motility and a decrease in sperm count. This effect is dose and duration dependent.

Conclusively, the aqueous leaf extract of *Hibiscus sabdariffa* has the potential to help in the management of male infertility due to Type 1 diabetes mellitus.

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