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https://dx.doi.org/10.4314/nvj.v45i1.5 **ORIGINAL ARTICLE**

Ameliorative Effects of Spondias Mombin Aqueous Leaf Extract on The Sperm Characteristics of Alloxan-Induced Diabetic Male Wistar Rats.

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SUMMARY

Considerable efforts have been directed towards addressing diabetes-induced infertility. In a two-phase study designed to determine the effect of Spondias mombin (SM) aqueous leaf extract on blood glucose, spermiogram and gonadosomatic index of diabetes-induced wistar rats, forty male Wistar rats (170 - 250g) were randomly divided into ten groups (n=4) with five treated for 7 days for the first phase and five treated for 14 days in the second phase. Each phase had their five groups randomly assigned into control (non-diabetic), diabetic (untreated), diabetic concurrently treated with 5 mg/kg glibenclamide, 400mg/kg SM and 800 mg/kg SM. Diabetes mellitus was induced with intraperitoneal administration of alloxan hydrate at a dosage of 158mg/kg. Rats with fasting blood glucose of ≥ 190 mg/dL three days post alloxan administration was considered diabetic. Following this, the test substances were administered daily via oral gavage for 7 or 14 days. Blood glucose levels, spermiogram, body weight changes, gonadosomatic index (GSI), and testicular weights were evaluated at the end of each phase. Results revealed that SM caused a reduction in the blood glucose in a dose-dependent manner in alloxan-induced diabetic rats. In both phases, 800 mg/kg SM dosage improved the sperm motility and concentration. Also, at 400mg/kg dosage, after 14 days of administration SM caused a progressive improvement in the sperm motility and concentration. The percentage of abnormal sperm cells varied between control and treatment groups but remained within the range of 18.50% to 57.00% after 14 days of treatment. The GSI was highest in 800 mg/kg SM group after 14 days when compared with other groups. This study concluded that the aqueous S. mombin leaf extract exhibited an ameliorative effect on diabetes-induced infertility when administered at dose of 800 mg/kg between 7 and 14 days.

Key words: Spondias mombin, Diabetes mellitus, Blood glucose, Semen characteristics, Infertility

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia resulting from lack of insulin secretion or resistance of host tissue to insulin (Omolaoye *et al.,* 2018). Clinical findings associated with DM are caused by uncontrolled hyperglycemia, leading to morbidity and mortality (Mohammed *et al.*, 2007). DM is one of the most frequently diagnosed endocrinopathies in dogs and cats in veterinary medicine, with reported incidence rates of 0.4-1.2% in the US and 0.22% in Nigeria (Nelson and Reusch, 2014; Gani and Ihedioha, 2015).

The global prevalence of infertility in diabetic men is currently about 50 % (Lotti and Maggi, 2023). This is because of a reduction of sperm quality and motility caused by the condition (Ryan and Gajraj, 2012; Bhattacharya *et al.*, 2014). Also, pancreatic insulin has been found to regulate the male hypothalamic-pituitary-gonadal axis, which is crucial for fertility (Loeken, 2012).

Although several therapeutic interventions have been adopted towards mitigating DM, such as alpha-glucosidase inhibitors, biguanides, thiazolidinediones etc (Alam *et al.*, 2018). Some draw backs associated with their use as therapy include obesity, vomiting, hypoglycaemia, etc. This has necessitated the search for safer alternatives for managing DM and its attendant anti-fertility effect (Alam *et al.,* 2018; Adekoya *et al.,* 2022). Recent research has shown that DM can impact sperm formation and androgen production, ultimately leading to male infertility (Sushma *et al.,* 2019). Diabetes mellitus affects male reproductive system at pre-testicular, testicular, post-testicular and histology levels.

Medicinal plants such as *Vernonia a mygdalina* (Adekoya *et al*., 2022), *Ocium gratissimum* (Akharaiyi *et al*., 2017), and *Psidium guajava* (Adeleye *et al*., 2022), have been documented as possessing antidiabetic activities made possible by their hypoglycaemic, anti-hypercholesterolemic and antioxidative properties thereby mitigating pathologies associated with DM (Adekoya *et al.*, 2022). *Spondias mombin* (SM) is a tree found in the rainforest and is known by various names across various languages in West Africa (Okwu and Okwu, 2004). The leaves contain saponins, tannins, alkaloids, and flavonoids (Igwe *et al.*, 2010). Traditionally, various part of SM are used for different medicinal purposes, including the treatment of diseases and as forage for domestic animals (Ayoka *et al.*, 2008). In south-eastern Nigeria, juice extracts from SM leaves are used to facilitate delivery in small ruminants with dystocia arising from uterine inertia, and it has been used in livestock for increased productivity (Mussarat *et al.*, 2014).

Administration of the aqueous extracts of *Spondias mombin* resulted in effective anti-hyperglycemic activity in alloxanized rats (Goodies *et al.,* 2015). Aqueous extract of *Spondias mombin* supported fertility in the male Wistar rat hence a good source of fertility enhancement in male animals (Oloye *et al*., 2011; Olufunke *et al*., 2014). Since Diabetes mellitus has been linked to male fertility problems (Omolaoye *et al.*, 2022), there is the need to provide for diabetic male patients a pro-fertility preparation that is not only effective but also cheap, easy to access and safe for consumption. The study, therefore, sought to investigate the probable ameliorative effect of the aqueous extract of SM leaves on the indices of fertility in diabetic male Wistar rats.

MATERIALS AND METHODS

Animals

Forty (40) healthy adult male Wistar rats weighing between 170g and 250g were used for the experiment. The rats were kept in the animal house of the Department of Veterinary Physiology and Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria. They were kept under standard condition (inverted 12h light/dark cycle) and maintained on standard feed ration with water given *ad libitum*.

Preparation of aqueous leaf extract of *Spondias mombin*

Fresh leaves of SM were obtained within the premises of the Federal University of Agriculture, Abeokuta, Ogun State of Nigeria. The leaves were authenticated at the National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan. The fresh leaves were air-dried and pulverized into powdery form. A measure of 520 g of the powder was soaked in a glass measuring cylinder with distilled water for aqueous extraction for 48 hours followed by filtration. The filtrate collected was taken through the process of evaporation in a water bath at 40^{o}C and the extract was stored at 4°C (Behravan *et al.*, 2019).

Induction of diabetes mellitus and treatment

The rats were randomly divided into five groups (I-V) consisting of eight animals each. Diabetes mellitus was induced in Groups II-V with alloxan hydrate (Kem® Light Laboratories, Pvt. Ltd., India) at a dosage of 158 mg/kg by intraperitoneal injection. Rats showing elevated fasting blood glucose levels above 190 mg/dl at 72 hours post-administration using a glucometer (Accu-Chek ®, Germany) were considered diabetic (Sherif *et al.*, 2015).

Experimental design

The study design was randomized controlled trial. The rats were randomly divided into ten groups (n=4). Five groups were treated for 7 days for the first phase of the study and the other five groups were treated for 14 days in the second phase. Each phase had their five groups randomly assigned as follows:

Group I: Control given distilled water (1 ml/kg)

Group II: Diabetic given distilled water (1ml/kg)

Group III: Diabetic treated with glibenclamide (5mg/kg)

Group IV: Diabetic treated with *Spondias mombin* (400 mg/kg)

Group V: Diabetic treated with *Spondias mombin* (800 mg/kg)

Body weight and Blood fasting Glucose

Daily body weights were recorded and at termination samples of blood were collected for fasting blood glucose measurement.

Testicular weight and Spermiogram

Following euthanasia of the Wistar rats via cervical dislocation, a midline scrotal incision was made to reveal the caudal epididymis from where semen was collected (Saba *et al.,* 2009). Testes were promptly removed, and rinsed in physiologic saline solution, blotted and weighed using a digital mini scale (Pocket digital mini scale).

Sperm motility: A drop of buffered semen was placed on a clean pre-warmed glass slide to which pre-warmed cover slip was applied and viewed at x400 magnification for gross motility of spermatozoa. Spermatozoa were considered motile when seen making a head-forward unidirectional progressive motility (Oyeyemi and Ajani, 2015).

Sperm morphology: Smears were prepared from the sperm sample and stained with Giemsa stain for identification of morphologically abnormal spermatozoa and to determine their percentages. (Oyeyemi and Ajani, 2015). The percentages were determined by identifying and counting the abnormal spermatozoa from a total count of 400 spermatozoa in the slides and express in percentage thereafter.

Sperm viability: Prepared sperm smears stained with pre-warmed eosin-nigrosin stain were observed under the microscope at x400 magnification, for percentage live-dead ratio evaluation (Saba *et al.,* 2009).

Sperm concentration: Spermatozoa concentration was estimated using the improved Neubauer Chamber (Deep 1/10mm, LABART, Germany) as described by Basiru and Olayemi (2014).

Gonadosomatic index determination: This was calculated based on the body and testicular weight, using the formula:

$$\text{GSI} = \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}}\ x\ 100$$

(Ramalan *et al.*, 2021)

Statistical analysis

Results were expressed as mean and standard deviation. The differences between means were analyzed statistically with one-way analysis of variance, followed by Tukey's comparison test using GraphPad prism 7.0 and a *p*-value ≤ 0.05 was considered to be statistical significant.

RESULTS

Effects of test groups on diabetic Wistar rats after 7 days of administration

A significant decrease ($p<0.05$) in the blood glucose level in the glibenclamide, *S. mombin* extract at 400 mg/kg and 800 mg/kg groups was observed following daily oral administration for 7 days of the study when compared with the untreated diabetic group (Figure 1). The *S. mombin* 800 mg/kg group was however found to be more effective when compared to *S. mombin* 400 mg/kg and glibenclamide group. Weight reduction was observed over the 7 days of treatment and this was statistically lower in treated groups when compared with the control group. The testicular weight was found to be highest in the control group, of note however was the significantly lower ($p<0.05$) testicular weight of

the glibenclamide group compared with all the test groups. The gonadosomatic index of *S. mombin* 800 mg/kg group was found to be the highest when compared to others, though not significant (Table 2).

Sperm motility in the glibenclamide, *S. mombin* 800 mg/kg and control groups differ significantly ($p<0.05$) and were higher when compared with the untreated diabetic and *S. mombin* 400 mg/kg groups. A similar trend was observed with percentage sperm livability where glibenclamide, *S. mombin* 800 mg/kg and the control groups had significantly higher values compared with the untreated diabetic and *S. mombin* 400 mg/kg groups (Table 2). Sperm concentration ranged from 76.75±29.65 to 175.25±19.57 and that of *S. mombin* 800 mg/kg group was significantly higher than that of glibenclamide group (Table 2) Percentage sperm morphological abnormality was lowest in the control group (24.25±4.15) and highest in the untreated diabetic group. There was a significant difference in the values obtained for control compared with untreated diabetic and *S. mombin* 400 mg/kg group. Also, a significant difference was observed comparing glibenclamide, untreated diabetic and *S. mombin* 400 mg/kg groups.

Effects of test groups on diabetic Wistar rats after 14 days of administration

After 14 days of treatment, the study revealed a significant lowering of blood glucose level in the *S. mombin* 400 mg/kg group when compared with the untreated diabetic group (Figure 1). A decrease in body weight was observed in the untreated diabetic, glibenclamide and *S. mombin* 800 mg/kg groups, however, an increase in body weight was seen in the *S. mombin* 400 mg/kg group after 14 days of treatment (Table 1).

The testicular weight was found to be highest in the control group and as it was for 7 days of administration the testicular weight of the glibenclamide group was significantly lower ($p< 0.05$) compared with all the test groups. The gonadosomatic index of *S. mombin* 800 mg/kg was observed to be higher compared with the rest of the groups (Table 2).

The *S. mombin* 800 mg/kg group had the highest sperm motility of 70.00±5.7 7 when compared with other treatment groups

The sperm viability was higher in the control group, untreated diabetic and *S. mombin* 800 mg/kg groups compared with glibenclamide and *S. mombin* 400 mg/kg groups, however there was no statistically significant difference ($p>0.05$) when comparing the trio of glibenclamide, *S. mombin* 400 mg/kg and *S. mombin* 800 mg/kg (55.00±14.43, 68.50±0.87 and 78.50±0.87) with the untreated diabetic group.

The *S. mombin* 400 mg/kg group had the significantly highest ($p<0.05$) spermatozoa concentration (167.00±16.74 x10^6cells/ml) compared with other groups. The percentage of morphological abnormality was between 18.50 and 57.00% in all the groups. The untreated diabetic group had the highest percentage of spermatozoa morphological abnormality (Table 2).

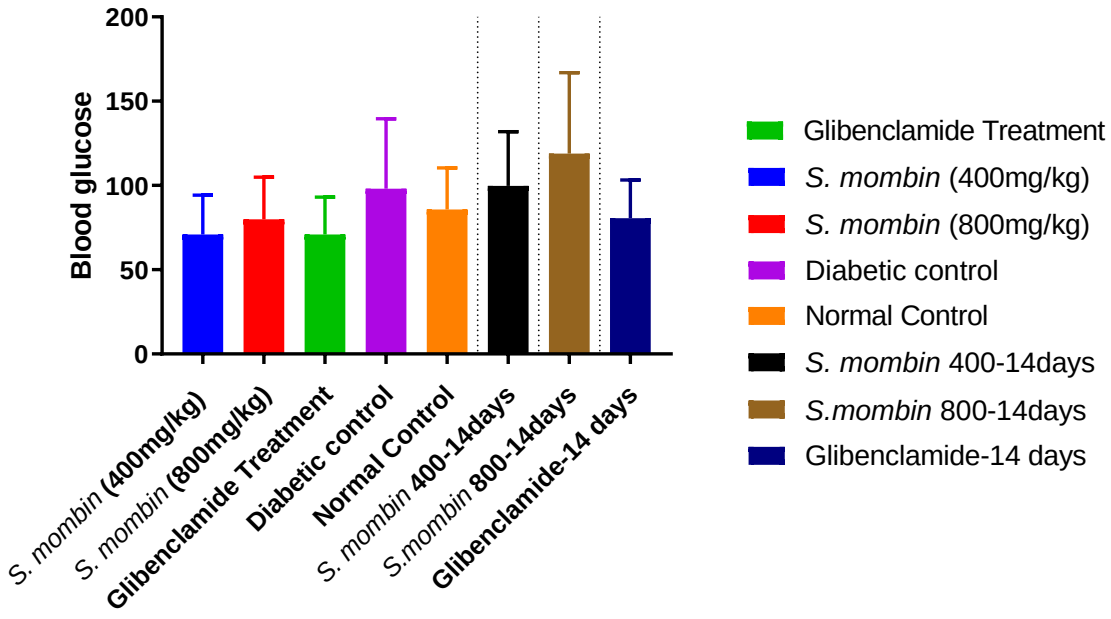

Figure 1. Effect of *Spondias mombin* leaf extract on the fasting blood glucose in alloxan-induced diabetic male Wistar rats after 7 and 14 days of treatment

Table 1. Effect of aqueous extract of *Spondias mombin* leaves on body weight changes in alloxan-induced diabetic male Wistar rats

	Weight Before (g)	Weight After (g)	Weight Change (g)
Normal control	200.50±2.02[a]	221.00±3.46[a]	20.50±1.44[a]
Diabetic control	198.00±0.00[a]	170.00±0.00[a]	-14.00±8.08[a]
Glibenclamide (7 days)	182.00±9.81[a]	158.00±9.24[a]	-24.00±0.58[a]
***S. mombin* 400mg/kg (7 days)**	192.50±3.18[a]	177.50±2.02[a]	-15.00±1.15[a]
***S. mombin* 800mg/kg (7 days)**	193.00±2.31[a]	191.50±6.06[a]	-1.50±3.75[a]
Glibenclamide (14 days)	172.00±0.00[a]	159.00±0.00[a]	-6.50±3.75[a]
***S. mombin* 400mg/kg (14 days)**	250.00±0.00[a]	271.00±0.00[a]	10.50±6.06[a]
***S.mombin* 800mg/kg (14 days)**	248.00±0.00[a]	218.00±0.00[a]	-30.00±0.00[a]

[a]Mean (±SD) values with same superscripts are not significantly different along column lines ($p > 0.05$)

Table 2: Semen parameters of alloxan-induced diabetic male Wistar rats treated with *Spondias mombin* leaf extract

	Sperm motility	Sperm livability (%)	Sperm concentration	Sperm abnormality	Testicular weight	GSI
Normal control	89.00±3.11^{a}	90.00±2.45^{a}	121.50±4.99^{a}	24.25±4.15^{b}	1.53±0.05^{a}	0.69±0.01^{a}
Diabetic control	35.00±5.00^{c}	30.00±10.00^{c}	91.00±21.00^{a}	56.50±2.50^{a}	1.20±0.00^{b}	0.65±0.00^{b}
Glibenclamide (7 days)	72.75±2.75^{a}	71.50±3.97^{a}	76.75±29.65^{a}	31.75±4.97^{b}	1.03±0.03^{c}	0.65±0.03^{b}
***S. mombin* 400mg/kg (7 days)**	38.75±11.97^{c}	38.75±11.97^{c}	121.25±7.09^{a}	55.00±8.28^{a}	1.18±0.05^{b}	0.66±0.02^{b}
***S. mombin* 800mg/kg (7 days)**	71.25±1.25^{a}	71.25±1.25^{a}	175.25±19.57^{a}	33.25±1.97^{b}	1.40±0.07^{a}	0.73±0.03^{a}
Glibenclamide (14 days)	65.00±5.00^{a}	65.00±5.00^{b}	146.50±35.50^{a}	57.00±11.00^{a}	0.75±0.25^{d}	0.47±0.16^{b}
***S.mombin* 400mg/kg (14days)**	50.00±10.00^{b}	50.00±10.00^{b}	167.00±29.00^{a}	33.50±6.50^{b}	1.60±0.00^{a}	0.59±0.00^{b}
***S. mombin* 800mg/kg (14days)**	70.00±10.00^{a}	70.00±10.00^{a}	104.50±6.50^{a}	32.50±3.50^{b}	1.45±0.05^{a}	0.67±0.03^{a}

^{a,b,c,d} Mean (±SEM) values with different superscripts significantly differ across columns ($p< 0.05$)

DISCUSSION

A dose dependent hypoglycemic effect was observed with the two *S. mombin* treatments (400 mg/kg and 800 mg/kg) after a 7 days treatment period. This finding agrees with Gobinath *et al.*, (2022), who evaluated the hypoglycaemic effect of methanol extract of the *S. mombin* in a glibenclamide model. This activity was suggested to be a resultant action of free radical scanvengers present in the plant as the flavonoid, tannins and polyphenol (Njoku and Akumefula, 2007). The

significant reduction in blood glucose levels by the plant extracts is attributed to increased insulin secretion and enhanced insulin sensitivity (Adebayo *et al.*, 2009).

In this study, a significant decline in the sperm characteristics were observed in the diabetic untreated group compared to the non-diabetic animals. Reports on decline in testicular function and spermatogenesis can result from diabetes mellitus (Type I and II) (Jain and Jangir, 2014) as was observed with the diabetic animals in this study, demonstrating the antifertility effect of DM in the male Wistar rats. Further observations from this study showed that the sperm parameters (sperm motility, sperm viability and sperm concentration) of diabetic rats after 7 days and 14 days treatment with 800 mg/kg *Spondias mombin* aqueous leaf extract were significantly enhanced. Also, a similar effect on sperm parameters following a 14 day treatment with 400mg/kg *Spondias mombin* aqueous leaf extract was observed. Martin and Touaibia (2020) reported in their review that plant extracts rich in flavonoids, such as those found in the leaves of *Spondias mombin*, can improve sperm parameters. These flavonoids could potentially influence the production of androgen (majorly testosterone) by the Leydig cells. (Murugesan *et al.*, 2005).

The absolute and relative weight of the testes of the two *Spondias mombin* treatments significantly improved when compared with diabetic controls following 7 and 14 days of treatment. The reduction in weight of testes is often attributed to decreased population of germ cells (spermatogonia), spermatids and spermatocytes at various stages (Kanter *et al.*, 2013). According to Gonzales *et al.* (2001), any procedure that increases the weight of the sex organs may have androgenic properties. Testicular organ weights are used as indicators of reproductive toxicity, and a reduction of testicular weight is a sensitive parameter for interpretation of male gonadal toxicity (Creasy, 2001). The improved gonadosomatic index could therefore be an indicator of pro-fertility property of the leaf aqueous extract of *Spondias mombin.*

CONCLUSION

This study has revealed that aqueous *Spondias mombin* leaf extract has ameliorative effect against diabetic induced infertility at 800 mg/kg for a 7 - 14 days treatment and at 400mg/kg when used only for 14 days. When using 800mg/kg for 14 days. Care must however be taken as the blood glucose level might be elevated.

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