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Evaluation of the effect of ethanol leaf extract of *Vernonia amygdalina* on the Testicle of Diabetic Wistar Rats

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Abstract

The aim of this study was to evaluate the effect of ethanol leaf extract of *V. amygdalina* on the testicle of alloxan induced diabetic wistar rats. Freshly harvested leaves of *V. amygdalina* was washed with clean tap water and subsequently dried at room temperature prior to being ground to fine powder. Twenty (20) adult male wistar rats were divided into four (4) groups of five (5) rats per group.

- Group I was the normal control and was fed rat chow and water
- Group II was induced diabetes without treatment, while
- Groups III and IV were diabetic rats administered with 100 mg/kg and 200 mg/kg bw of *V. amygdalina* extract respectively.

Sperm morphology, motility and count evaluated indicated that the aforementioned parameters were significantly ($P<0.05$) low in diabetic rats which were not treated but however improved in a dose dependent manner to values which though were significantly ($P<0.05$) lower than that of the normal control. In conclusion, this study shows that extract of *V. amygdalina* can ameliorate damage imposed on the testicle by diabetes mellitus.

Keywords: *Vernonia amygdalina*; Sperm morphology; Diabetes; Sperm motility; Sperm count

1. Introduction

In Nigeria, bitter leaf (*Vernonia amygdalina*) popular known as olugbu among the Igbos of the South Eastern Nigeria is one of the most respected, valued and widely accepted vegetables within the region and has been reportedly ranked the most wildly cultivated specie of the genus *vernonia* with a height of approximately 1-3 m tall and having petiolatedly shaped green leaves with a diameter of about 6 mm [1]. The renowned bitter taste of the leaf accounts for its common name “bitter leaf” [2].

Research has shown that bitter leaf is an embodiment of valuable compounds such as saponins, flavonoids, alkaloids, and tannins etc [3]. Extract of *V. amygdalina* subjected to hydrodistillation revealed the presence of linoleic acid and oleic acid which are both essential [4] as well as α-pinene and eucalyptus linalool [5].

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Notably certain parts of *V. amygdalina* such as the root and leaf are reportedly employed in the treatment of gastrointestinal disorders, renal impairments, hiccups and fever etc. Its potential to ameliorate oxidative stress has also been established [6]. The ability of its extract to kill plasmodium is also known [7] and numerous therapeutic significances of the plant have been unveiled.

The most essential organ tasked with sexual and reproductive functions is the testes also know as testicle which is known to deliver the male sex gamete and hormone known as androgen. Type 1 diabetes is an autoimmune disorder characterized by the inability of the $^{\beta}$-cells of the pancreas to produce insulin which in turn translates to arrays of systemic effects on the body's metabolism one of which being reproductive dysfunction [8]. Research affirms that the efficacy of extracts of *V. amygdalina* is dose dependent and may deliver toxicity when employed to enhance diminished fertility [9]. Thus, it is imperative to investigate its effect on this very essential organ "Testicle".

2. Material and Methods

2.1. Collection and Processing of Plant Material

Fresh leaves of *V. amygdalina* harvested from a farm within the university campus in Uli, Ihiala Local Government Area of Anambra State was subsequently identified and authenticated at the herbarium unit of the Department of Botany, Nnamdi, Azikiwe University Awka, Anambra State. The leaves were well washed with clean tap water and afterwards dried at room temperature. The dried leaves were subsequently ground and sieved to fine powder.

2.2. Animals

Wistar rats used in this experiment weighed 150-200 g and were held in transparent plastic cages, housed in the Animal House of the Department of Human Physiology, College of Health Sciences, Anambra State University. The rats were fed rat chow and water *ad libitium*. They were acclimatized for three weeks.

2.3. Extraction of Plant Material

Precisely 400 g of the powdered leaf sample was steeped in 1L of 70% ethanol in an airtight conical flask for a period of 72 hr. The mixture was shaken intermittently at room temperature. The mixture was filtered firstly, through a double layered muslin cloth and then through Whatman No. 1 filter paper, the resulting residue was evaporated to dryness with the aid of a rotary evaporator under reduced pressure at 40-50°C [10].

2.4. Median Lethal Dose 50% (LD50)

The median lethal dose of extract was determined using three groups of three rats per group which were separately administered with 10, 100 and 1000 mg/kg of extract orally. The rats were studied for 24 hr to note any sign of toxicity. Following the absence of mortality in any of the groups, another three groups of one rat each were each administered with 1600, 2900 and 5000 mg/kg of extract separately. The animals were observed for 48 hr for signs of toxicity [11].

2.5. Induction of Experimental Diabetes

Induction of diabetes was performed by intraperitoneal administration of 150 mg/kg bw of alloxan. After 48 hrs animals with blood glucose level above 120 mg/dl were selected and used for the study [12].

2.6. Animal Grouping

- Group I: was fed with rat chow and water *ad-libitum*.
- Group II: diabetic induced rat without treatment
- Group III: diabetic rat administered with 100 mg/kg of *V. amygdalina* leaf extract.
- Group V: diabetic rat administered with 200mg/kg of *V. amygdalina* leaf extract.

2.7. Animal Sacrifice and Collection of Sample

2.7.1. Sample Collection

Animals were subjected to chlororoform anaesthesia, after which their abdomen was opened by a midline abdominal incision in order to reveal the reproductive organs. The harvested testicle was ridded fat and blood sample collected via cardiac puncture [13].

2.7.2. Sperm Motility

With the aid of a pipette, fluid obtained from the caudal epididymis was diluted to 0.5 mL with tris buffer solution. An aliquot of the solution was dropped on a slide placed on a light microscope with heater table. Percentage motility was evaluated at the magnification of ×400. Sperm motility was determined on three different fields for each sample which were relied upon to determine the final motility score [14].

2.7.3. Sperm Morphology

From the original dilution for motility, caudal sperm was taken and diluted 1:20 with 10% neutral buffered formalin. Score for morphological abnormalities was performed on 500 sperms. Spermatozoa were categorized transiently in wet preparation with the aid of the phase contrast optics. Spermatozoon with a rudimentary tail and round head was considered morphologically abnormal and was expressed as percentage of morphologically normal sperm [9]. The sperm morphology was evaluated with the aid of a light microscope at ×400 magnification.

2.7.4. Sperm Counts

The reproductive status of the rats was determined by performing epididymal sperm count. A small aliquot of a dilution of 10 mL NaCl 0.9% in pieces of caudal epididymal tissue was placed on a slide and examined with the aid of a light microscope. The Malassez's cell to count the number of spermatozoids in five randomly selected quadrants. Sperm count was determined using the formula below:

$$\text{Sperm count } = \frac{\boldsymbol{X} \text{ x d}\boldsymbol{f} \text{ x } 10^6}{4}$$

% Viability= (Alive sperm/Dead sperm) x 100
% Mobility = (Mobile sperm/Total number of sperm) x 100
X = sperm count in 4 randomly selected quadrants of the Malassez's cell
df = dilution factor (20)

2.8. Histological Analysis

Table 1 Effect of Ethanol Extract of *V. amygdalina* Leaf on the Sperm Morphology of Sperm obtained from Diabetic Wistar rats

		Sperm Morphology	
Grouping	**Treatment**	**Normal**	**Abnormal**
Group I	Feed and water	85.00±2.88^{d}	15.00±2.88^{a}
Group II	Diabetic rats	40.00±5.77^{a}	60.00±5.77^{d}
Group III	D+100 mg/kg BLE	61.67±3.64^{b}	38.33±3.64^{c}
Group IV	D+200 mg/kg BLE	80.00±2.88^{c}	20.00±2.88^{b}

Values are expressed as mean ± standard deviation of three determinations

Table 2 Effect of Ethanol Extract of *V. amygdalina* Leaf on Sperm Motility of Sperm obtained from Diabetic Wistar rats

		Sperm Motility	
Grouping	**Treatment**	**Normal**	**Abnormal**
Group I	Feed and water	86.67 ± 1.67^{c}	13.33 ± 1.67^{a}
Group II	Diabetic rats	53.33 ± 6.01^{a}	46.67 ± 6.01^{c}
Group III	D+100 mg/kg BLE	70.00 ± 10.00^{b}	30.00 ± 10.00^{b}
Group IV	D+200 mg/kg BLE	70.33 ± 5.77^{b}	29.67 ± 5.77^{b}

Values are expressed as mean ± standard deviation of three determinations

Testicle was fixed in Bouin liquid and subsequently dehydrated and suspended in xylene for 2 hr and 30 minutes. The tissue was then embedded in paraffin and was cut to a thickness of 5 µm and subsequently, mounted on slides and stained by steeping in Mayer hematoxylin solution and afterwards rinsed with water to get rid of excess hematoxylin. The slides were dipped in alcohol, eosin solution and then dehydrated through a series of graded alcohols. Lastly, the tissue was mounted under a synthetic resin. Microscopic evaluation of the slides was undertaken and variations in histoarchitecture of the said organ noted [15].

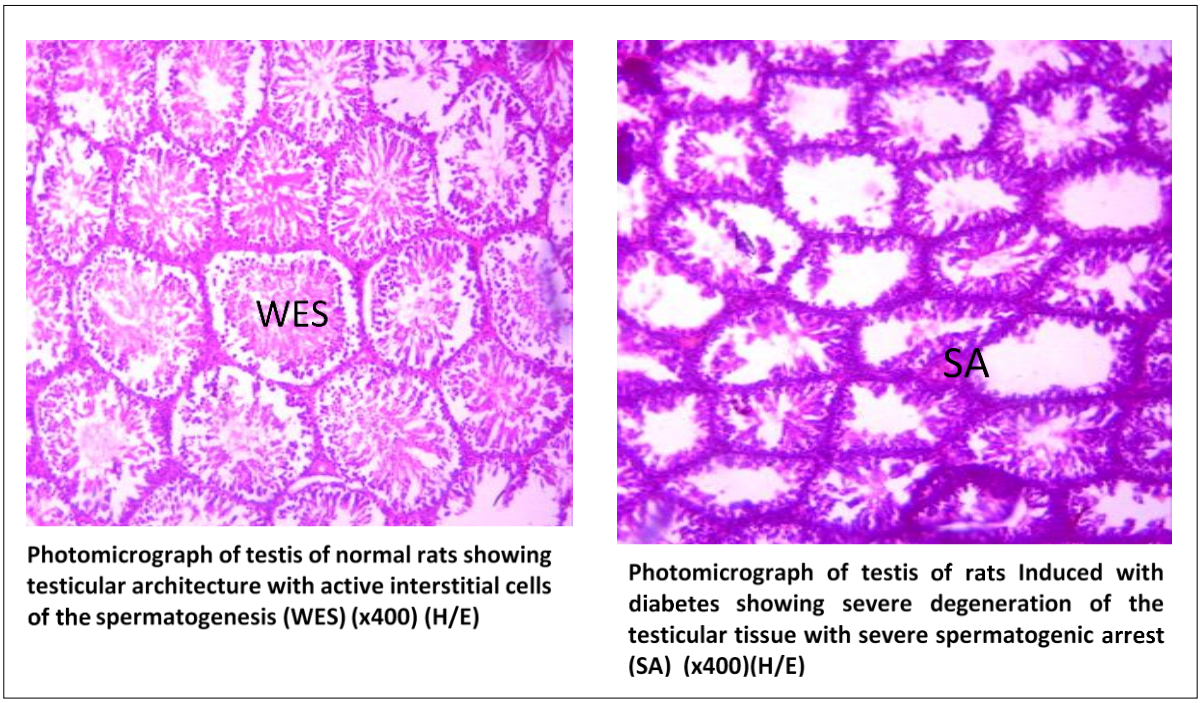

Figure 1 Testicle of Apparently Healthy and Diabetic Rats

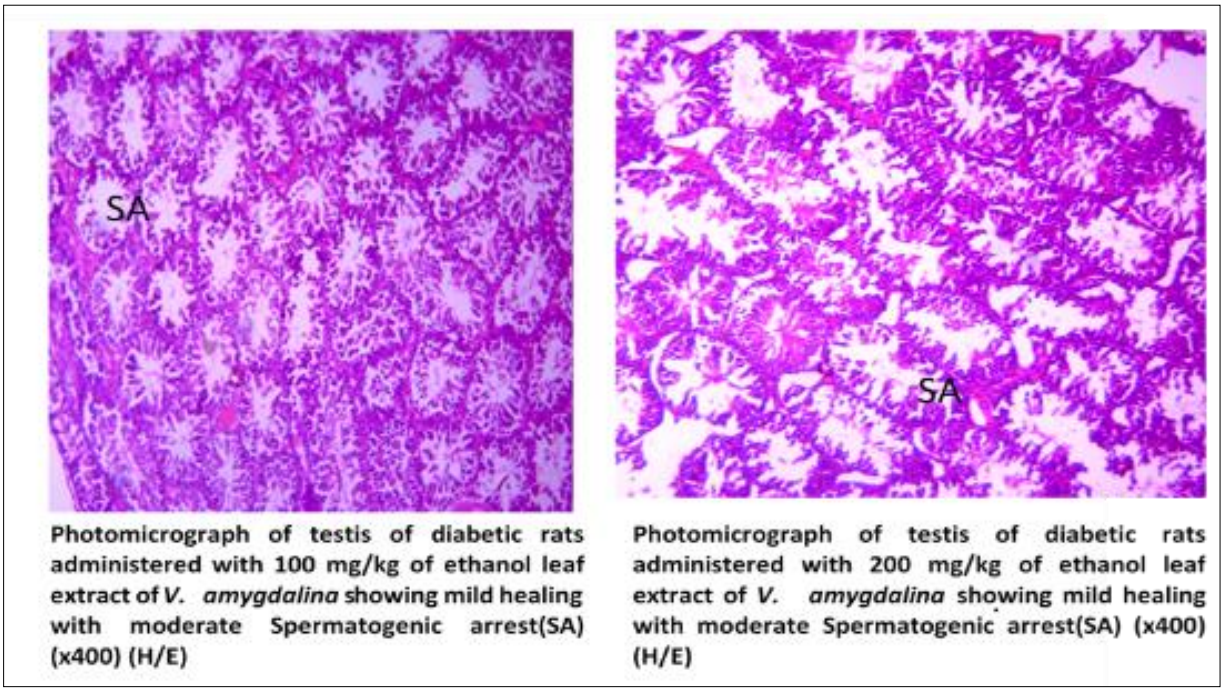

Figure 2 Testicle of Diabetic Rats treated with Varying Doses of Ethanol Leaf Extract of *V. amygdalina*

Table 3 Effect of Ethanol Extract of *V. amygdalina* Leaf on Sperm count of Sperm obtained from Diabetic Wistar rats

Grouping	Treatment	Sperm Count (× 10^6/mL)
Group I	Feed and water	636.67 ± 5.48^d
Group II	Diabetic rats	286.00 ± 3.00^a
Group III	D+100 mg/kg BLE	448.33 ± 68.46^b
Group IV	D+200 mg/kg BLE	495.00 ± 4.37^c

Values are expressed as mean ± standard deviation of three determinations

3. Results and Discussion

Type 1 diabetes is an autoimmune disorder characterized by the inabilityof the $^\beta$-cells of the pancreas to produce insulin which in turn translates to arrays of systemic effects on the body's metabolism one of which being reproductive dysfunction [8]. Bitter leaf has unique nutritional and phytochemical properties which has numerous physiological, biochemical and morphological benefits [16]. Table 1 shows the effect of ethanol leaf extract of *V. amygdalina* (Bitter leaf) on the morphology of sperm obtained from diabetic rats. 40.00±5.77% of sperm obtained from diabetic rats which were not treated with Bitter Leaf Extract (BLE) was normal which was however significantly (P<0.05) lower than the values reported for diabetic rats treated with 100 mg/kg and 200 mg/kg BLE which had 61.67±3.64% and 80.00±2.88% normal sperm which were however significantly (P<0.05) lower than the value reported for the normal control group. Table 2 shows the effect of ethanol leaf extract of *V. amygdalina* on the motility of sperm obtained from diabetic rats. The values reported for the motility of sperm obtained from diabetic rats which were not treated with BLE (53.33±6.01%) was significantly (P<0.05) lower than the values reported for diabetic groups treated with 100 mg/kg BLE (70.00±10.00%) and 200 mg/kg BLE (70.33±5.77%) both of which were significantly (P<0.05) lower than the value reported for the control group. Table 3, shows the effect of ethanol leaf extract of *V. amygdalina* on sperm count of sperm obtained from diabetic rats. While a significantly (P<0.05) lower sperm count was reported for diabetic rats which were not treated with BLE, results revealed a dose dependent increase in sperm counts in rats treated with BLE which however was significantly (P<0.05) lower than the value reported for the normal control group which had only feed and water. The significantly (P<0.05) lower percentage normal sperm, motility and count reported for diabetic rats could be attributed to a reduction in the sex hormones (luteinizing hormone [LH] and follicle stimulating hormone [FSH]) released by the pituitary gland in diabetic rats, which accounts for decreased testosterone levels [15]. This result is consistent with the finding of Ballester et al [16] which showed that the testicular sperm count, motility and testicular weight was pointedly reduced in diabetic rats, while the ameliorative effect of BLE on the parameters of sperm obtained from diabetic rats could be attributed to the potential of the aforementioned vegetable to increase glucose metabolism leading to the production of pyruvate which is known to be a preferred substrate for the activity and survival of sperm cells [16]. In addition, the flavonoids and vitamins inherent in bitter leaf extract could maintain sperm morphology and function [9]. This consistent with the finding of Saalu et al [9] which showed that administration of different doses of bitter leaf extract for 30 days elicited a dose dependent increase in sperm concentration, percentage motility, morphology and percentage live sperm**.**

4. Conclusion

Considering that previous research efforts had revealed that *V. Amygdalina* leaf extract, may be toxic when used to improved diminished fertility in a dose dependent manner, the outcome of this study, therefore projects *V. Amygdalina* as a very promising alternative to its synthetic counterparts in improving reproductive and sexual functions having established that the doses used productively ameliorated diabetes induced testicular damage.

Compliance with Ethical Standards

Acknowledgments

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Disclosure of conflict of interest

Authors hereby declare that no conflict of interest exists.

Statement of ethical approval

Animal use and care guidelines were strictly observed in accordance with the institution's committee on animal care and handling.

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