IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 14, Issue 3 Ser. II (May – June 2019), PP 84-88
www.Iosrjournals.Org

Methanolic Leaf Extract of Vernonia Amygdalina on the Testes of Streptozotocin Induced Diabetic Wistar Rats.

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Abstract: *This study aimed at investigating the effects of methanolic leaf extract of Vernonia amygdalina (VA) on the histology of the testes of streptozotocin (STZ) induced diabetic Wistar rats. Thirty male albino Wistar rats were divided into groups of six rats each for this study. Group A (Normal Control) and B (Diabetic Control) that received 0.5 ml dimethylsulphoxide (DMSO) only. Group C received 5i.u/kg body weight of insulin, Group D received 400mg/kg b.w of VA and Group E received 75mg/kg b.w of flavonoid rich fraction of VA. Group B,C,D and E animals were induced for diabetes by intraperitoneal injection of 40mg/kg b.w of STZ reconstituted in 0.5M sodium citrate buffer. After 28 days, the animals were sacrificed and testes collected for histopathological studies. Histological observations of the testes showed normal testes with prominent seminiferous tubules containing germ cells at different stages of development and spermatozoa in Group A. In Group B, signs of degeneration in seminiferous tubules, destruction of germ cells, spermatozoa and Leydig cells was observed. In Groups C,D &E the testes showed normal seminiferous tubules with Leydig cells, sertolic cells and active spermatogonia cells at various stages of maturation suggestive of possible regeneration. Methanolic leaf extract and flavonoid rich fraction of VA showed marked improvement on diabetic testes*

Key words: *Vernonia amygdalina, diabetes, spermatozoa*.

Date of Submission: 10-06-2019 Date of acceptance: 26-06-2019

I. Introduction

Evidence has shown that some herbal remedies are highly efficacious, potent with minimal side effects and highly affordable thus the need to carry out researches in this area (Odugbemi, 2008). *Vernonia amygdalina* (VA) commonly known as bitter leaf exist as a shrub of about 2-5nm with petiolate leaf of about 6mm diameter and elliptic shape. It grows under a range of ecological zones in Africa, produces large mass of forage and is drought tolerant (Hutchinson and Dalziel, 1963). Its liquid form used as an extract has diverse application in ethno-medicine ranging from treatment of parasitic infections, (Philipson *et al.* 1993: Izerbelic *et al.*, 2004), Breast cancer (Wong *et al.*,2013), diabetes *mellitus* (Okolie *et al.*, 2008) and induction of gastric secretion (Owu *et al.*, 2008). In its macerated form it is consumed as vegetables and condiments for soup. In this form it can also serve as the cheapest source of vitamins, minerals and essential amino acid (Okafor, 1983). Through research and studies, phytochemical analysis of the leaves of VA yielded two known sesquiterpene lactones: vernolide and vernodalol (Sani *et al*; 2012). Other active constituent include– essential oil, tannins, alkaloid, saponins, glycosides, anthraquinones and flavonoids all of which may participate in herb-drug interaction. These phytochemicals are believed to be responsible for the myriad medico-biochemical activities of this plant, acting singly or synergistically to produce the result for which *Vernonia amygdalina* is known. The testes is one of the target organ in many cases of diabetic complications as about 90% of diabetic male patients have disturbances in sexual function, including a decrease in libido, impotence and infertility (Jiang *et al;* 1996), more commonly, consistent inability to achieve and maintain penile erection sufficient for adequate sexual relations, or Erectile dysfunction (Rehman *et al;* 2001) (De Young *et al;* 2004). Research has it that diabetes causes infertility in animal models. Testicular weight, sperm quantity and quality, and testosterone production were affected negatively (Koh, 2007) (Ricci *et al;* 2009). This study was aimed at determining the effects of methanolic fraction of *Vernonia amygdalina* on the testes of streptozotocin induced diabetic Wistar rats.

II. Materials And Method

Thirty male albino Wistar rats were divided into groups of six rats each for this study. Group A (Normal Control) and B (Diabetic Control) received 0.5ml dimethylsulphoxide (DMSO) only. Group C received 5i.u/kg body weight of insulin, Group D received 400mg/kg b.w of VA and Group E received 75mg/kg b.w of flavonoid rich fraction of VA. Group B,C,D and E animals were induced for diabetes by

intraperitoneal injection of 40mg/kg b.w of STZ reconstituted in 0.5M sodium citrate buffer. After 28 days, the animals were sacrificed and testes collected for histopathological studies.

III. Results

Histological appearance of the testes was demonstrated using H and E staining technique as represented in photomicrographs with the following observations. Group A (control) showed sections of the normal rat testes with prominent seminiferous tubules consisting of proliferating spermatocyte at various stages of maturation. These include primary spermatocytes, secondary spermatocytes and spermatids.Their lumen are filled with spermatozoa and the intervening interstitium contains round to oval leydig cells (Plate 1). Group B (diabetic control): sections showed seminiferous tubules containg spermatocytes and the lumen found to be almost occluded. These cells are enlarged with prominent nuclei and distinct cell membrane, their chromatin pattern are coarse.The cells are mainly primary spermatocytes.The basement membranes (BM) are intact (Plate 2). Group C (insulin treated): the seminiferous tubules are prominent containing developing spermatocytes at various stages of maturation closely related to Group A. The intervening interstitium is scanty containing leydig cells and the basement membrane is intact with cell layers thick (Plate 3). Group D (Crude VA): sections here shows prominent seminiferous tubules with intact basement membrane consisting of spermatocytes at various stages of maturation in contrast to the ones in Group B. The cells are layers thick with regular cellular outline and oval to round nuclei.The lumen shows sign of recanalization as compared to the diabetic control group and contains flagellae of spermatozoa. The intervening interstitium are scanty and contain leydig cells (Plate 4). Group E (flavonoid-rich fraction of VA): showed sections of the testes having prominent seminiferous tubules with intact basement membrane consisting of spermatocytes at various stages of maturation.The cells are layers thick.Their lumen contains flagellae of spermatozoa. The intervening interstitium are scanty and contain leydig cells (Plate 5).

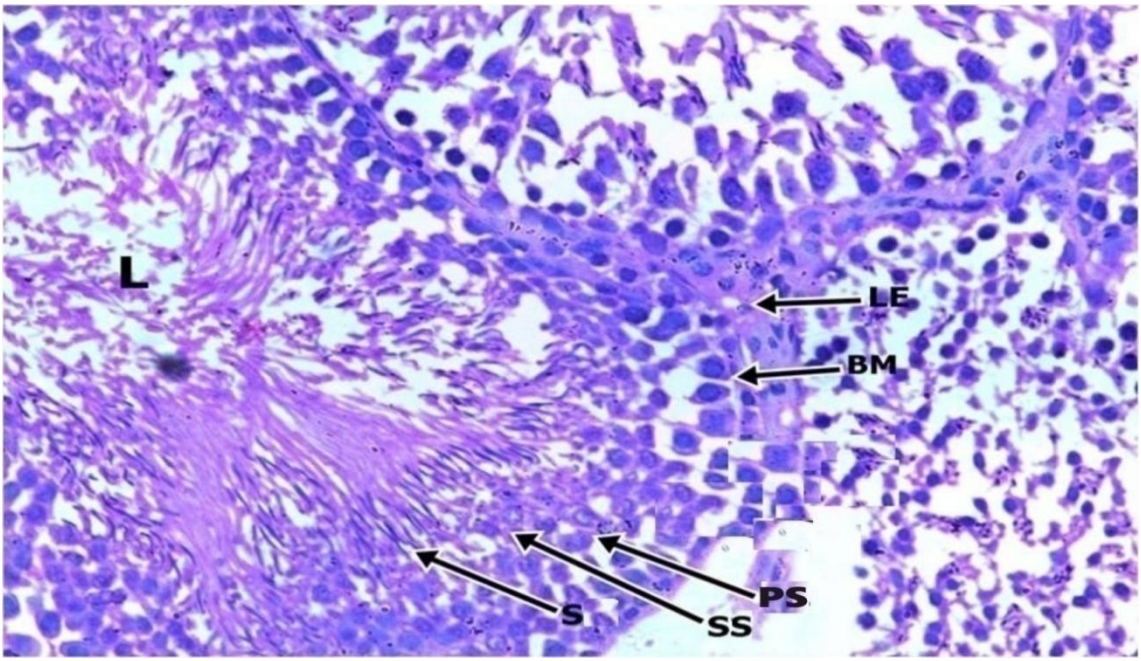

PLATE 1: PHOTOMICROGRAPH (X400) OF TESTIS OF NORMAL CONTROL RATS IN GROUP A. Haematoxylin and eosin stain

These include primary spermatocytes (PS), secondary spermatocytes(SS) and spermatids (S).Their lumen (L) are filled with spermatozoa and the intervening interstitium contains leydig cells (LE)

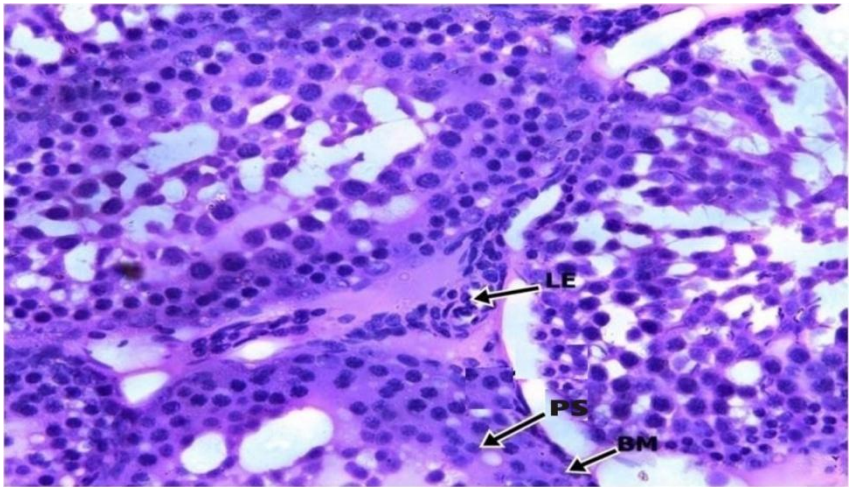

PLATE 2: PHOTOMICROGRAPH (X400) OF TESTIS OF DIABETIC CONTROL RATS IN GROUP B Haematoxylin and eosin stain

These cells are enlarged with prominent nuclei and distinct cell membrane, their chromatin pattern are coarse.The cells are mainly primary spermatocytes (PS).The basement membranes (BM) are intact.

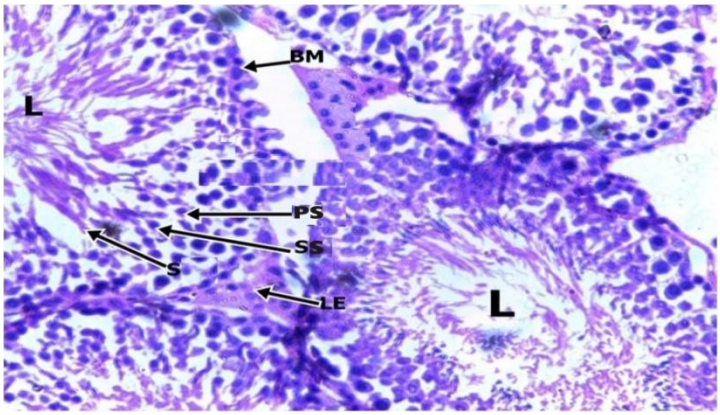

PLATE 3: PHOTOMICROGRAPH (X400) OF TESTIS OF INSULIN TREATED RATS IN GROUP C Haematoxylin and eosin stain

The seminiferous tubules are prominent containing developing spermatocytes at various stages of maturation of primary spermatocytes (PS), secondary spermatocytes (SS) and spermatids (S). The intervening interstitium is scanty containing leygig cells and the basement membrane is intact with cell layers thick

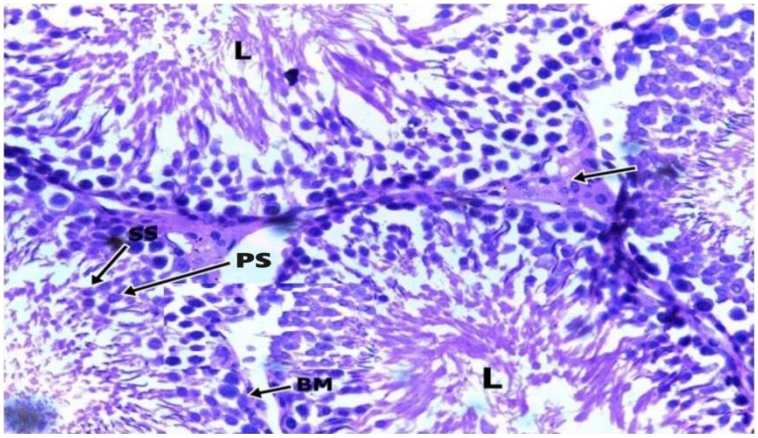

PLATE 4: PHOTOMICROGRAPH (X400) OF TESTIS OF CRUDE EXTRACT OF VA TREATED RATS IN GROUP D Haematoxylin and eosin stain

Prominent seminiferous tubules with intact basement membrane consisting of spermatocytes at various stages of maturation of primary spermatocytes (PS), secondary spermatocytes (SS) and spermatids (S).

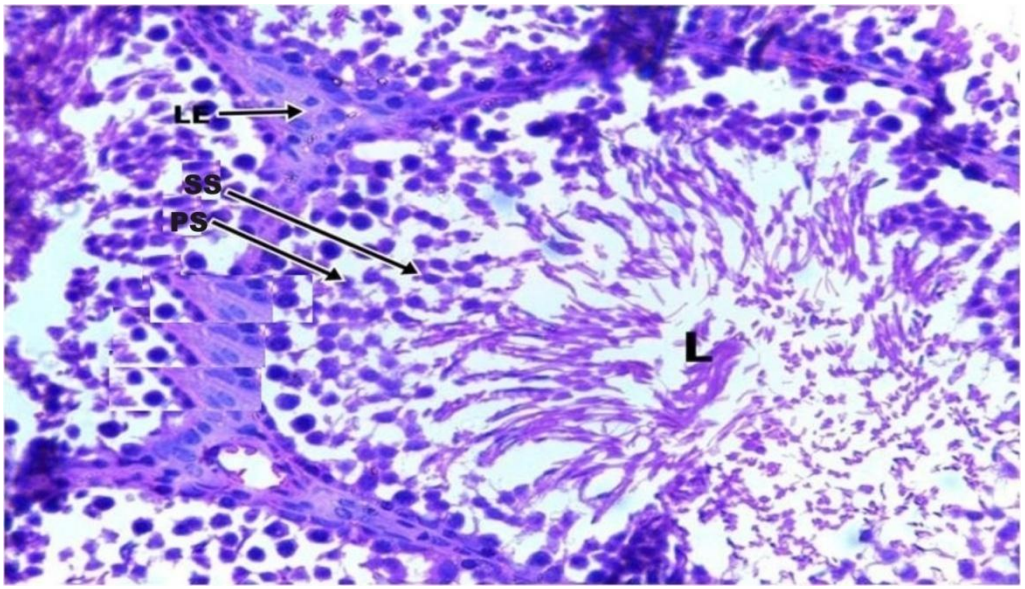

PLATE 5: PHOTOMICROGRAPH (X400) OF TESTIS OF 30 % METHANOL FRACTION OF VA TREATED RATS IN GROUP E Haematoxylin and eosin stain

Sections of the testes having prominent seminiferous tubules with intact basement membrane consisting of spermatocytes at various stages of maturation (primary spermatocytes (PS), secondary spermatocytes(SS) and Leydig cells (LE)

IV. Discussion

The effect of methanolic leaf extract of *Vernonia amygdalina* on the histology of the testes of streptozotocin induced diabetic Wistar rats was investigated as research and studies have shown that the leaf extract of VA has hypoglycemic properties in experimental animals and could be useful in the management of diabetes mellitus (Ong *et al*, 2011). In the diabetic control, the seminiferous tubules showed signs of degeneration with decrease in seminiferous tubuli diameter with narrowed lumen, few spermatozoa present, Leydig cells were also reduced. Ozturk *et al*. (2002) reported that STZ induced diabetes is associated with atrophy of seminiferous tubules, destruction of spermatogenic cells and thickening of basal membranes. Thickening of the basal membranes hinders the nourishment of the impaired seminiferous tubules resulting in impaired spermatogenesis. Baries *et al*. (2009) also reported germ cell degeneration, vacuolation of seminiferous tubules, damaged and atrophied seminiferous tubules with intertubular spaces filled with fluid on alloxan induced diabetic rats. In the groups that received crude and 30% methanol fraction of VA (flavonoid – rich fraction), there was marked improvement in the histology of the testis as lumen showed signs of recanalization, and regeneration of testicular. The tissue sections appeared normal with Leydig cells and germ cells at different stages of development similar to that of the control group. Spermatozoa were present in the lumen of the seminiferous tubules and interstitial tissues appeared normal. This is in cognizant with earlier studies which reported the potentials of extracts of *Vernonia amygdalina, Gongronema latiforium and Azadirachta indica* to cause regeneration of pancreatic beta cells of STZ induced diabetic rats (Ebong *et al* 2006; Akpaso *et al*., 2011). Akpaso *et al*., (2017) also reported possible regeneration of testicular cells and a reversal of diabetic insults on the cells following combined methanolic leaf extracts of *Vernonia amygdalina, Gongronema latiforium* on sperm parameters and testes in STZ induced diabetic Wistar rats. Fischer and Fischer (2017) reported increase in the serum concentration level of testosterone following administration of methanolic leaf extract of VA on STZ induced diabetic male Wistar rats. These restorative effects so witnessed can be ascribed to the bioactive components of the leaf extract of *Vernonia amygdalina* which contains phytochemicals (flavonoids) rich in antioxidants. Flavonoids have been reported to possess antioxidant, anti-allergic, anti-inflammatory, anti-microbial and anticancer activities (Edeoga *et al*., 2005). Flavonoids amidst other properties has the capacity to act as antioxidant and this is exhibited in its antiatherosclerotic effect as few clinical studies have pointed out that flavonoid intakes protect against coronary heart disease (Hertog *et al*, 1995) which is a major diabetic complication thus improved antioxidants status forms one of the mechanism by which dietary antioxidants contribute to the prevention and reduction of diabetic complications.

V. Conclusion

Vernonia amygdalina contains flavonoids rich in antioxidants. Antioxidants improves spermatogenesis, intercept and prevent effects of oxidative stress on spermatozoa thus ameliorating testicular damage as seen in this study. *Vernonia amygdalina* can therefore be useful in reversing diabetic insults on the structure of the testes and consequently improve male fertility

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IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.

Victor A. Fischer " Methanolic Leaf Extract Of Vernonia Amygdalina On The Testes Of Streptozotocin Induced Diabetic Wistar Rats." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 14.3 (2019): 84-88.