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EFFECT OF ETHANOLIC LEAF EXTRACT OF *MUCUNA PRURIENS* ON THE TESTES OF ALLOXAN-INDUCED DIABETIC MALE WISTAR RAT

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ABSTRACT

Objective: This research study was carried out to investigate the effect of ethanolic leaf extract of *Mucuna pruriens* (*M. pruriens*) on the histology of the testes of alloxan-induced diabetic wistar rats.

Methodology: Twenty-five (25) male wistar rats weighing 200-250g were procured and acclimatized for two weeks, after which they were divided into seven (7) groups of five (5) rats each, and were housed in cages. The groups were designated as groups A, B, C, D and E. Groups B-E were induced with diabetes using alloxan. Groups A and B served as control groups and received only distilled water; while groups C – D diabetic served as the experimental groups and were given Glucophage, 400mg/kg of *M. pruriens* and 800mg/kg of *M. pruriens* respectively for 21 days through oral route with the aid of oral gastric tube. On the 22nd day, the animals were sacrificed via chloroform inhalation, and testes were harvested for histological studies.

Result: There were severe testicular damage with severe spermatogenic arrest, severe apoptosis of the interstitial cell of leydig and severe fatty change within the lumen of the animals in group B when compared with the control group A and group C that received water and glucophage respectively. These effects were ameliorated in Groups C - D which received variable doses of the ethanolic leaf extracts ***M.*** *prurines.*

Conclusion: The leaf extracts of *M. pruriens* have ameliorative effect on the testes alloxan-induced Wistar rats.

Keywords: *Mucuna purines*, diabetes, testes.

1.0 INTRODUCTION

Diabetes induces increasing oxidative stress to the male reproductive system [1]. Also, it causes many functional and structural syndromes and complications in organs, such as testis, brain, heart and retina [2, 3, 4]. Humans with diabetes have lower levels of spermatogenesis, sperm count, sperm motility, seminal fluid volume and testosterone when compared to healthy ones [5, 6, 7]. Impotence, degenerate fertility characterized by abnormal sperm, retrograde ejaculations, and erectile dysfunction are seen in diabetes [1]. Thus the urgent need to find out local herbs that will not only be accessible and affordable to all level of income earners, but will also ameliorate diabetes and its complications on the testes since the average age of patients suffering from diabetes-induced reproductive damage is getting younger.

About 422 million people worldwide have diabetes, the majority living in low-and middle-income countries, and 1.6 million deaths are directly attributed to diabetes each year [8]. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades [8]. Access to affordable treatment, including insulin, is critical for the survival of people living with diabetes. There is a globally agreed target to halt the rise in diabetes and obesity by 2025 [8]. Thus, the need to research on local herbs that will not only be accessible and affordable to all level of income, but will also ameliorate diabetes and its complications.

Diabetes can be said to be a chronic, metabolic disease characterized by increased levels of blood glucose (or blood sugar), leading to over time serious damage to the heart, blood vessels, eyes, kidneys and nerves [8], and is characterized by thirst, polyuria, blurring vision and weight loss. High blood sugar damages organs and tissues in the body [9]. The higher the blood sugar is and the longer one live with it, the greater the risk for complications associated with diabetes [9]. Such complications include: heart disease, heart attack, stroke, neuropathy, nephropathy, retinopathy, vision loss, hearing loss, foot damage, skin conditions such as bacterial and fungal infections, depression and dementia [9]. Its most common is type 2 diabetes which usually occurs in adults when the body becomes resistant to insulin or does not make enough insulin. In the past three decades, the prevalence of type 2 diabetes has risen dramatically in countries of all income levels. Type 1 diabetes, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin by itself [8].

Medicinal plants are used with the intention of maintaining health, to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine [10, 11]. They may provide health benefits to the people who consume them as medicines; financial benefits to people who harvest, process, and distribute them for sale; and society-wide benefits, such as job opportunities, taxation income, and a healthier labour force [11]. *Mucuna pruriens* is one of such plants.

M. pruriens whose English common names are monkey tamarind, velvet bean, Bengal velvet bean, Florida velvet bean, Mauritius velvet bean, Yokohama velvet bean, cowage, cowitch, lacuna bean, and Lyon bean; is a tropical legume native to Africa and tropical Asia and widely naturalized and cultivated [12]. It is notorious for the extreme itchiness for which it produces on contact [13], particularly with the young foliage and the seed pods; and also produces many medium-sized red swollen bumps along with the itching. It parts possess valuable medicinal properties and has been proven to have anti-diabetic effect, aphrodisiac, anti-neoplastic, anti-epileptic, and anti-microbial activities [14], anti-venom activities [15],

neuroprotective effect [16], anti-helminthic activity [17], analgesic and anti-inflammatory activity [18]. Also, it has proven to have antidiabetic effect through its ameliorating effect on the serum levels of testoerone, follicle stimulating hormone, insulin and growth hormone on alloxan induced male wistar rats [19].
The roots, leaves and seeds of the plant are commonly used in the treatment of impotence, snake bite, diabetes, cancer and Parkinsonism [20]. It has an affinity for all of the tissues in the body, but is especially suited to balance the nervous, reproductive, and digestive systems [21]. Research has shown that it can minimize the effects of stress while supporting the body's natural physical balance and good posture, a healthy gait, as well as fluid muscular movements, proper sensation, coordination, and keen motor skills [22]. It contains naturally occurring levodopa (L-dopa) - an essential precursor of the neurotransmitter dopamine which plays an important role in behavior, cognition, voluntary movement, sleep, mood, working memory, and learning [23].

M. pruriens is also an aphrodisiac, bolstering healthy sexual energy and libido [21]. It also supports normal fertility, healthy sperm and ova, proper functioning of the reproductive organs, and appropriate genital secretions [21, 24]. Thus it supports every aspect of *shukra dhatu* (the reproductive tissue) and as such it is being referred to both men and women [21].

Thus, this research study was carried out to investigate the effect of *M. prurines* on the testes of alloxan-induced diabetic wistar rats since no work has been carried out on this.

2.0 MATERIALS AND METHODS

2.1 Animal procurement, care and treatment

Twenty five (25) male wistar rats weighing between 160g to 200g were procured and housed at the Animal house of Anatomy Department, Abia State University, Uturu with wire gauze cages in a well-ventilated area. They were fed with standard commercial pellet diet and water *ad libitum*. There were acclimatized for two weeks before the experiment. Their health statuses were closely monitored before and during the experiment. All procedures were carried out in strict accordance with the Institutional guidelines on the care and use of experimental animals.

2.2 Collection, identification and preparation of plant material

Fresh leaves of *M. pruriens* were harvested from a local settlement in Uturu, Isuikwuato Local Government Area of Abia state. The leaves were properly washed with water to remove sand and other impurities, and were authenticated at the herbarium of the Department of Physiology and Pharmacology, Department of Forestry, College of Natural Resources and Environmental Management, Micheal Okpara University of Agriculture, Umudike. Voucher number was assigned to the identified plant as MOUAU/VPP/17/017. They were air dried and crushed using laboratory blender. Extraction was done using ethanol. The crude ethanol extracts were filtered into a stainless basin with a white cloth and placed in a water bath so as to dry up the ethanol. 250mg of these extracts/kg body weights were dissolved in 10mls of distilled water and administered to the animals.

2.3 Induction of diabetes

The rats were divided into non-diabetic control group and experimental groups. The baseline blood glucose level of the experimental group to be inducted was determined before the induction of diabetes. The rats were allowed to fast over night prior to injection of alloxan and diabetes was induced by intra-peritoneal administration of 150mg of alloxan per kg body weight

of rat (150mg/kg body weight). After the induction, the rats were allowed to have free access to the same feed and water. After 72 hours, blood samples obtained through tail tip puncture of the rats were used to confirm diabetes in the rats by testing for hyperglycemia using Glucometer. Diabetes was confirmed at fasting blood glucose levels greater than 200mg/dl [25].

2.4 Experimental protocol

The animals were grouped into five (5) groups of five (5) rats each. Different doses of the leaf extracts were administered via oral route with the aid of oral gastric tube as shown below:

Group A (The Control group) distilled water.
Group B (Diabetic group) distilled water.
Group C Diabetic + Glucophage
Group D Diabetic + 400mg/kg of *M. pruriens* leaf extract.
Group E Diabetic + 800mg/kg of *M. pruriens* leaf extract.

2.5 Sample collection and analysis

The extracts were administered for twenty one (21) days. On the 22nd day, the animals were sacrificed by anaestethizing under chloroform vapour and dissected. Testes organs were harvested from the animals, and were fixed in 10% formal saline for four hours. This was followed by histological and histochemical methods of tissue processing.

3.0 RESULTS
Histopathological findings

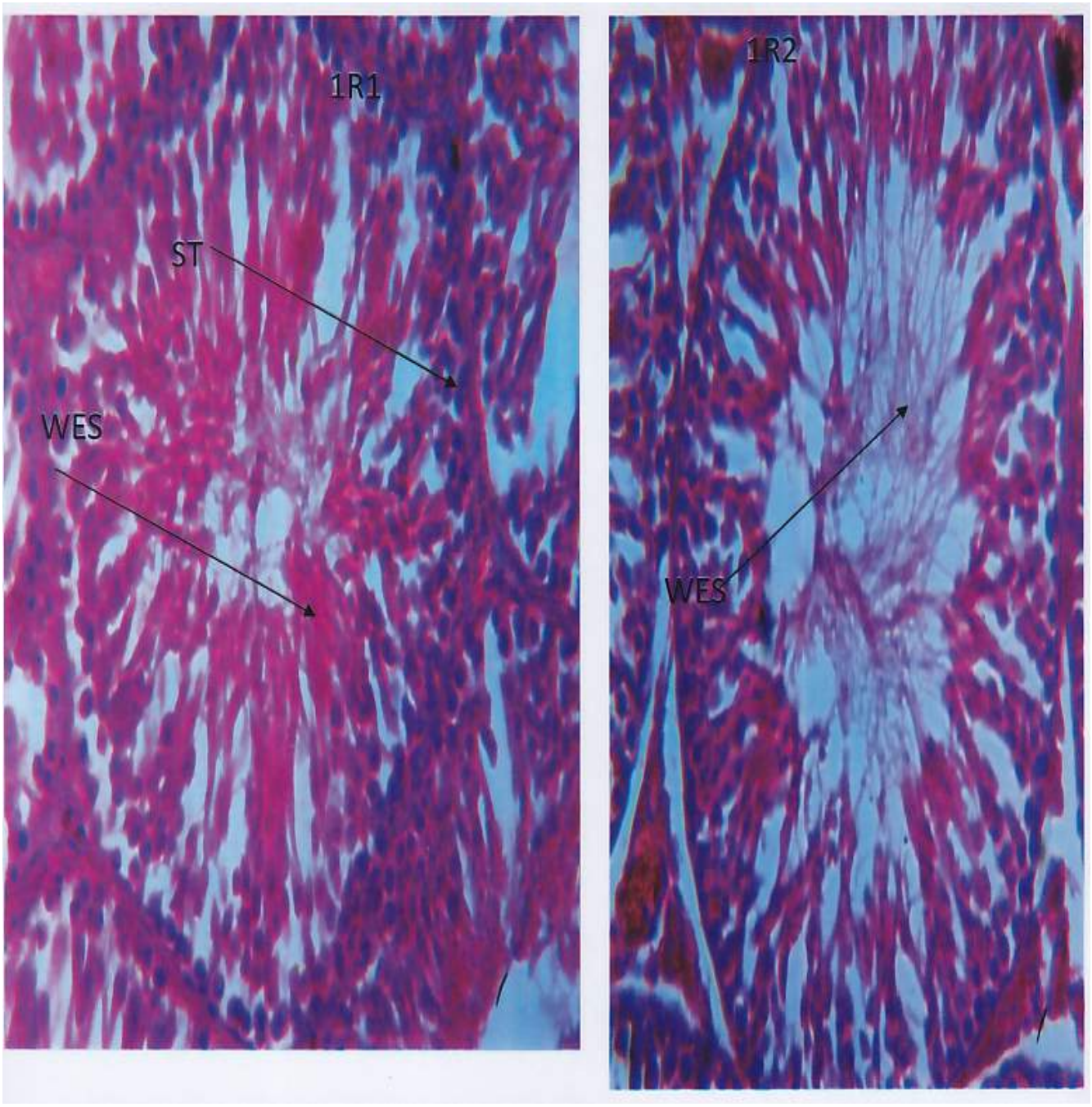

Figure 1: Histopathological analysis of wistar rat testes: (A) Control(x400) (H/E)

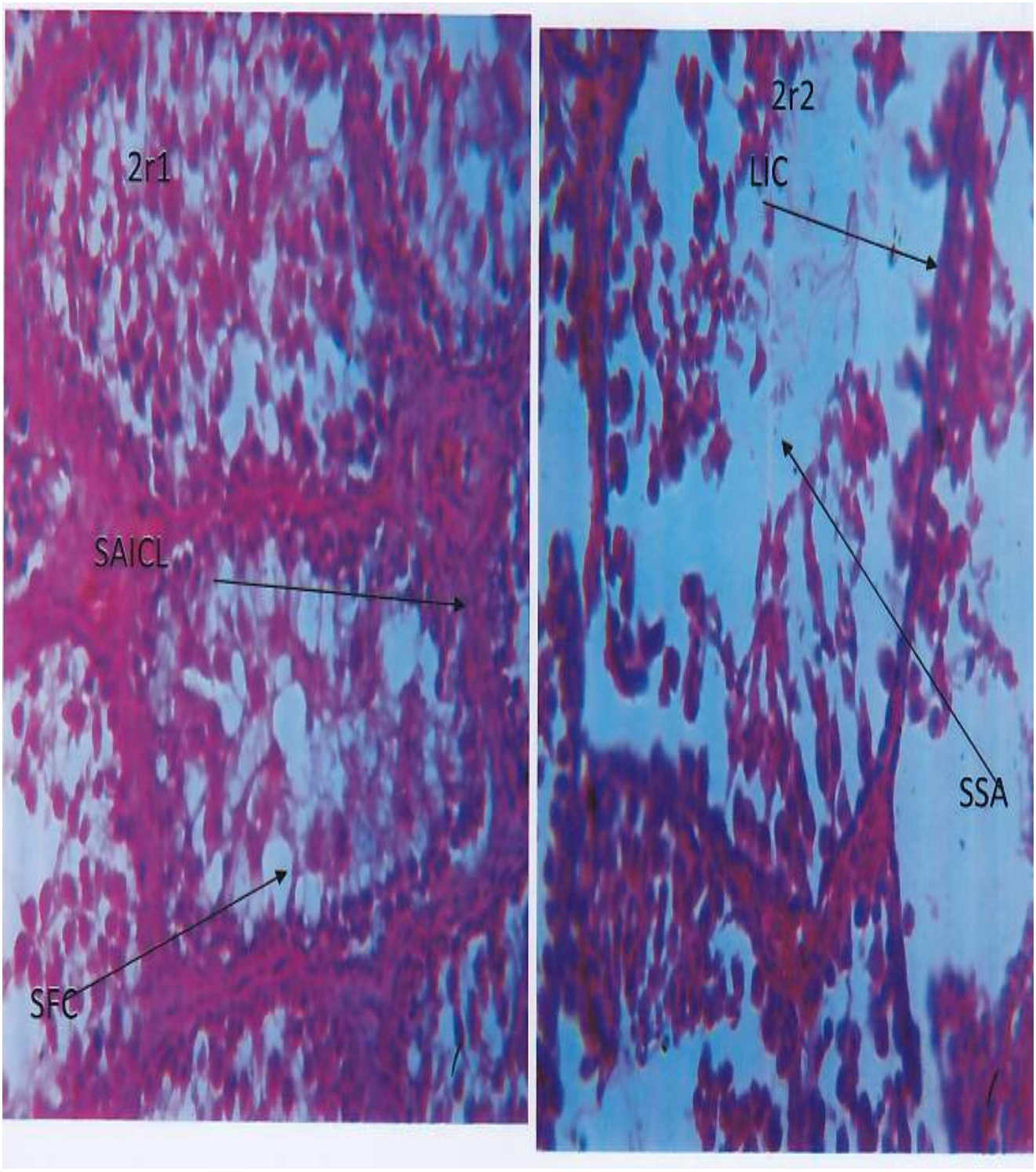

Figure 2: Histopathological analysis of wistar rat testes: (B) alloxan 150mg/kg of body weight(x400) (H/E)

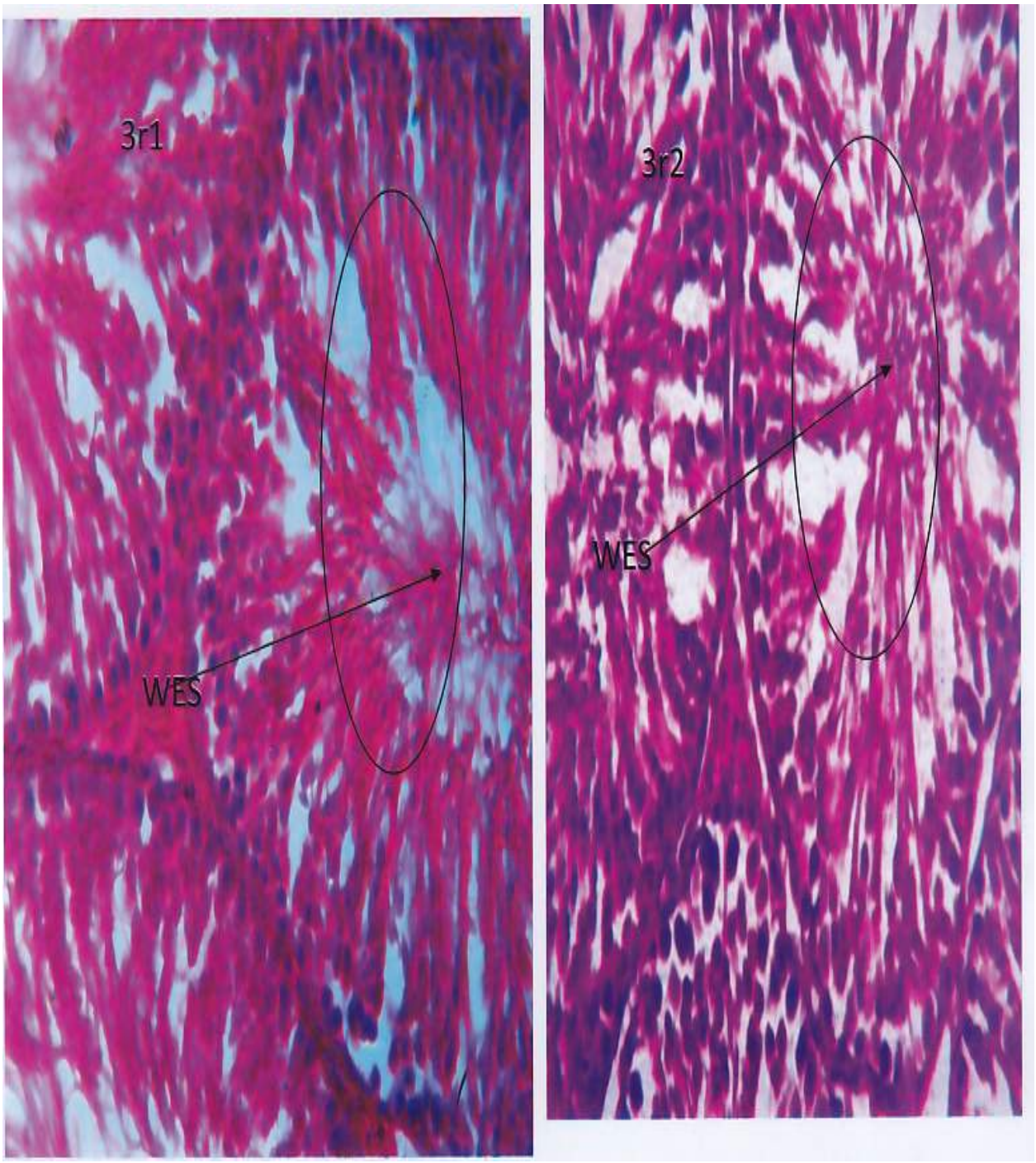

Figure 3: Histopathological analysis of wistar rat testes: (C) glucophage with one day spacing (X150) (H/E)

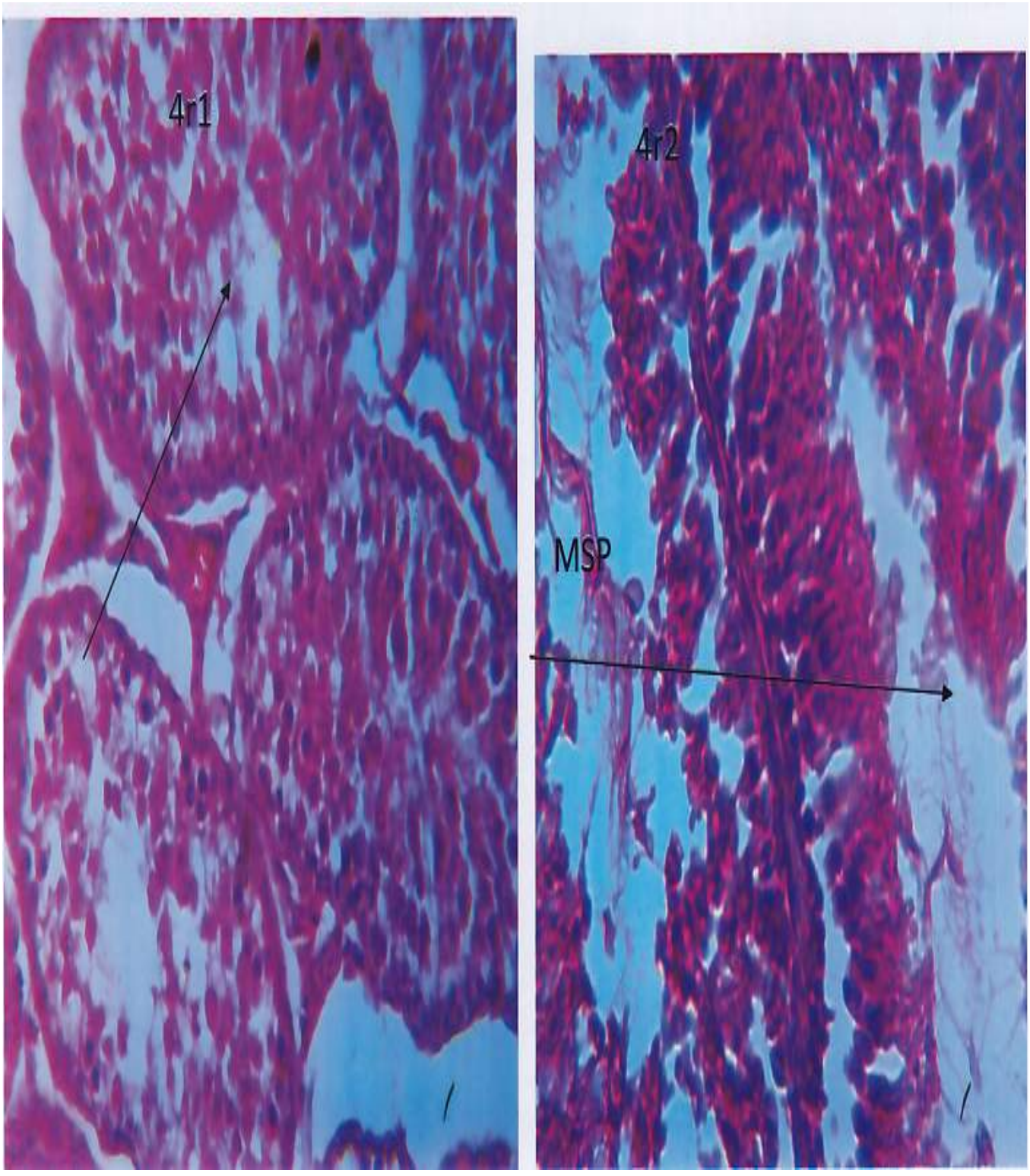

Figure 4: Histopathological analysis of wistar rat testes: (D) 400mg/kg of *M. pruriens* (x400) (H/E)

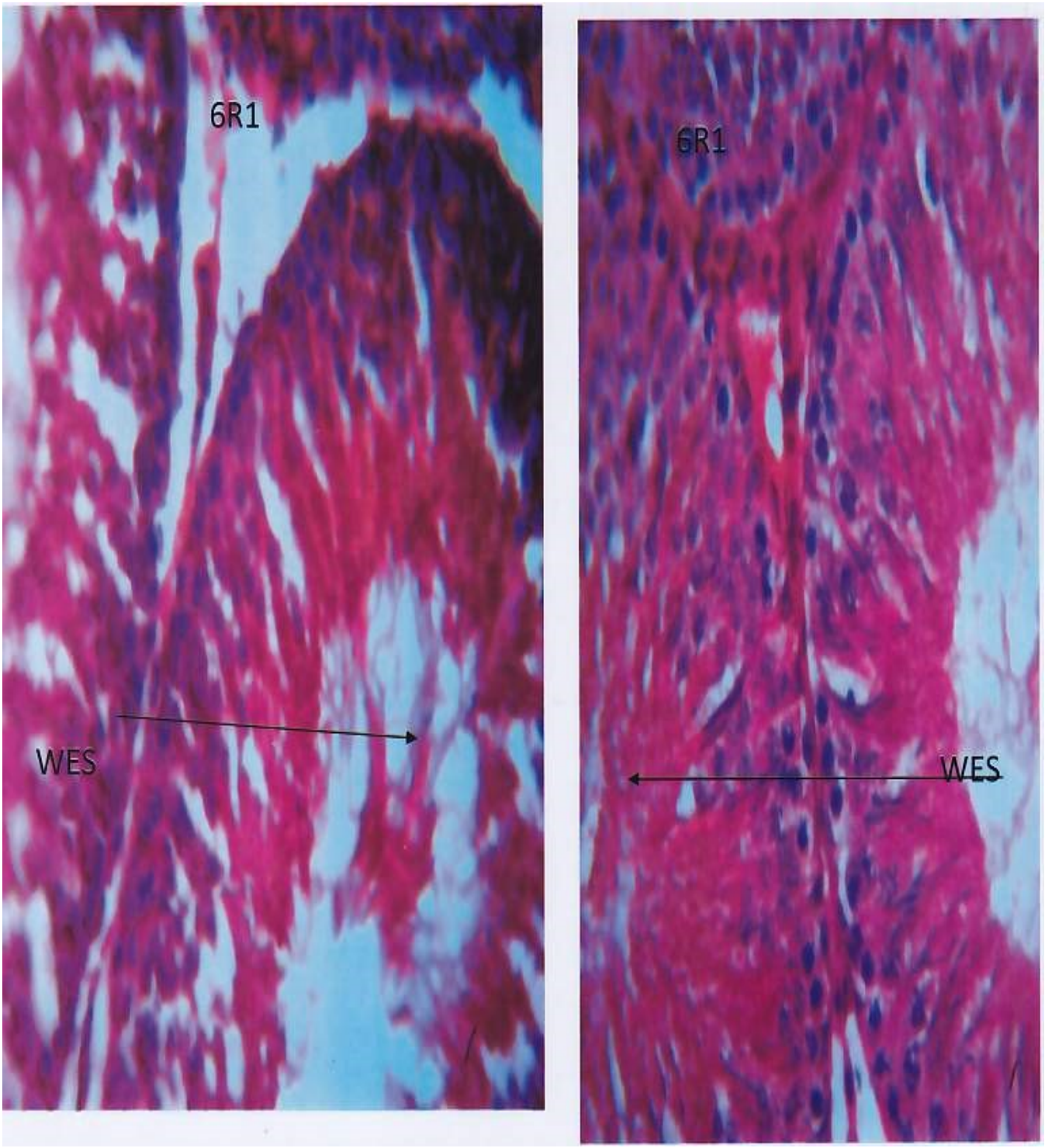

Figure 4: Histopathological analysis of wistar rat testes: (D) 800mg/kg of *M. pruriens* (x400) (H/E)

4. DISCUSSION

DM is a pathological condition of hyperglycemia which could result in damage to multiple organs and systems, including male reproductive system dysfunction. Type 2 DM is characterized by absent responsiveness of body tissues to insulin, namely, insulin resistance. In this study, the histopathological findings of testicular cells of the wistar rats in group (A) showed normal testicular architecture with semniferous tubule that are lined with interstitial cells of the leydig and well enhanced spermatogenesis (WES).Thus the overall feature appears normal (figure 1), while that of figure 2 (experimental group B) showed severe testicular damage with severe spermatogenic arrest (SSA), severe apoptosis of the interstitial cell of leydig (SAICL) and severe fatty change (SFC) within the lumen. These effects in figure 2 could be due to oxidative stress which impaired testicular DNA, survival of reproductive cells and function of spermatogenesis leading to the male infertility [26, 27]. Also hyperglycemia due to the induced alloxan could cause damage in multiple organs and systems, including male reproductive system dysfunction since Type 2 diabetes mellitus (T2DM) is characterized by absent responsiveness of body tissues to insulin, namely, insulin resistance and can easily induced long-term complications which lead to destruction of small blood vessels, include damage to eyes, nerves and testes [28, 29, 30].

Figure 3 treated with glucophage with one day spacing showed well regenerated testicular tissue with well enhanced spermatogenesis (WES). The healing effect in fig.3 was due the fact that Glucophage (Metformin) belongs to the class of medications called oral hypoglycemics, which are medications that lower blood sugar. It is used to control blood glucose (blood sugar) for people with type 2 diabetes. It is used when diet, exercise, and weight reduction have not been found to lower blood glucose well enough on their own. It works by reducing the amount of glucose made by the liver and by making it easier for glucose to enter into the tissues of the body and has been found to be especially useful in delaying problems associated with diabetes for overweight people with diabetes [31].

Figure 4 and 5 treated with 400mg/kg and 800mg/kg of *M. pruiens* showed mild regeneration with mild spermatogenesis progression (MSP) and well regenerated testicular tissue with well enhanced spermatogenesis (WES) respectively. These could be due the antidiabetic and ameliorating effects of the leaf extract of *M. pruiens* to the testicular tissue [14, 20, 19]. The result of this finding also confirmed that *M. pruriens* leaf just like its seed has an anti-hyperglycemic action, and could be a source of hypoglycemic compounds [32]. Its effect is better and well pronounced at higher dose of 800mg/kg.

5. Conclusion

This study has shown that ethanolic leaf extract of *M. pruriens* has antidiabetic and ameliorating effect on the testes of alloxan-induced male wistar rats. Thus, this therefore supports the use of the leaf of *M. pruriens* for the treatment of diabetes mellitus.

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Conflict of interest: None declared.
Ethical Approval: Approved by Institutional ethical approval.

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