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RESEARCH ARTICLE

Histological Evaluation of the Effects of *Vernonia amygdalina* (Bitter Leaf) Extract on the Pancreas of Alloxan-induced Diabetes in Adult Albino Wistar Rats

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Abstract

Diabetes mellitus is a chronic, complicated illness with numerous crippling side effects, most of which have multiple underlying causes. The purpose of this investigation was to examine the anti-diabetic properties of *Vernonia amygdalina* leaf extracts on the microstructure of the pancreas of adult Wistar rats following alloxan-induced diabetes. A total of thirty (30) albino Wistar rats were used for this experiment. The rats were divided into six groups; Group 1=normal control, Group 2= negative control, Group 3=low dose treatment group, Group 4= medium dose treatment group, Group 5= high dose treatment group and Group 6= Vitamin E (50mg/kg, oral) as the standard drug group. The negative control group was induced with alloxan (1.5 ml/kg) body weight only while the low, medium and high dose groups were administered 0.5 ml/kg, 1.0 ml/kg and 1.5ml/kg respectively of ethanolic extract of *Vernonia amygdalina* for fourteen days and induced with alloxan (1.5 mg/kg) and for seven days. At the end of the experiment, pancreas toxicity was evaluated histo-pathologically by light microscopy, data from the measurement of body weight were statistically analyzed using SPSS version 23 package. The negative control group showed acini metaplasia and focal area of acini aplasia. The other

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experimental group's pancreas showed normal acini with septa. General tissue appears normal. In the body weight studies, the changes between the negative control group, the treatment groups and standard drug group were statistically significant (P<0.05). Hence, it was established that *Vernonia amygdalina* (bitter leaf) has pancreaticoprotective effect against alloxan-induced diabetes in pancreas.

Keywords: *Vernonia amygdalina*, Diabetes Mellitus, Pancreas, Alloxan-Induced Diabetes.

1. Introduction

The plant, *Vernonia amygdalina* (bitter leaf) is found throughout Africa's west coast, where it grows both wild and as a home browsing plant.[1, 2] Because of the astringent, bitter taste of the leaves and stem, it is commonly referred to as "bitter leaf" in Nigeria. It is a key ingredient in Nigeria's well-known "bitter leaf soup" and has long been used in traditional medicine, especially by sub-Saharan Africans.[1, 3]

Among the people of Southern Nigeria, V. amygdalina has a good reputation for usage in the traditional therapy of diabetes mellitus. In an ethno-botanical investigation which identified and documented 22 plants of the South Western Nigeria used by traditional healers in the management of diabetes mellitus, V.amygdalina ranks the list as the most commonly used.[4]

Scientific studies have also reported its antihyperglycemic and hypoglycemic actions in diabetic rats.[5-7] The aqueous leaf extract has been proven to possess antihyperlipidemic and hypolipidemic action correspondingly on diabetic and non-diabetic rats. [8,9] . Its protective role on the kidneys[10] and livers[11] of alloxan diabetic rats has also been studied, with findings documented.

Diabetes mellitus is a chronic, complicated illness with numerous crippling side effects, most of which have multiple underlying causes. Since diabetes has several pathophysiological lesions, it would take more than one medication to reverse all or most of the disease's symptoms.

A multimodal therapeutic strategy is necessary for optimal treatment, and because a single herb contains a multitude of active chemicals, various traditional medicinal herbs have been chosen in this regard.[4, 10, 12, 13] Reactive oxygen species (ROS) production is known to be linked to diabetes mellitus, which impacts the antioxidant response that ROS scavenging enzymes catalyse and causes tissue damage.

The study by Bayraktutan[14] established the deficiencies in ROS scavenging enzymes in diabetes mellitus. A plant extract that has the ability to alleviate or reverse pancreatic lesions that results in hypoglycemia would by one method or the other address the ROS production or procedure. This implies that these plants need to have antioxidant qualities that would either mop up the ROS in the bloodstream or reverse the cytotoxic cycle of alloxan in the pancreas.

Diabetes is a complicated metabolic disease that is linked to subclinical inflammation, elevated oxidative stress, aberrant glucose and lipid metabolism, poor insulin signaling, and β-cell dysfunction. These metabolic diseases result in long-term pathogenic illnesses such as neuropathy, retinopathy, nephropathy, and micro- and macrovascular problems. As a result, quality of life declines and the rate of death increases.[15] Diet is the primary modifiable factor among the several risk factors that contribute to the occurrence and development of diabetes. Consuming vegetables high in phenolic compounds and having a high antioxidant capacity may have an inverse association with the incidence and prevalence of diabetes, according to both experimental and epidemiological research.[16, 17]

From the perspective of human medicine, the pancreas is a particularly significant organ since it is afflicted by two significant illnesses: pancreatic cancer and diabetes mellitus. At least 30 million individuals worldwide suffer from diabetes, which is still a serious issue even with the availability of insulin.[18] In order to reverse all or most of the disease's characteristics, more than one pharmacological agent would be needed because diabetes' pathophysiology involves many lesions. A multimodal therapeutic strategy is necessary for optimal treatment, and because a single herb contains a multitude of active chemicals, various traditional medicinal herbs have been chosen in this regard.[19]

Since the beginning of human history, medicinal plants have served as the foundation for healthcare worldwide, and they are still used today for a variety of chemotherapeutic reasons in both developed and developing countries. Therefore, this study is designed to provide a more available alternative to alleviate the harmful effects, thereby improving pancreatic function and antidiabetic qualities of V. amygdalina and to look into the mechanism underlying the antihyperglycemic action that has been documented, at least partially. The purpose of this study is to examine the effects of Vernonia amygdalina leaf extracts on the microstructure of the pancreas in adult Wistar rats after they have been given alloxan to induce diabetes.

2. Materials and Method

2.1 Collection and Identification of Plant Materials

Fresh leaves of *V. amygdalina* were gathered in the Etsako-west Local Government Area of Edo State, Nigeria, and verified by the Medicinal Plant Research and Traditional Medicine Department of the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja. A voucher specimen (No. NIPRD/H/15487) was deposited at the NIPRD Herbarium

2.1.1 Preparation of Extract

After being cleaned, the bitter leaves were allowed to air dry for a week at room temperature in an open area of the laboratory. After the drying process was over, the bitter leaf was removed and ground into a dry, smooth powder in a commercial blender. Both aqueous (water) extraction (WE) and ethanolic extraction (EE) were made using the fine powder (FP). Additionally, the bitter leaf extract was made utilizing the methanol extraction method, as previously detailed by Bawa *et al*.[18] After 100 grams (100 g) of the powdered leaves were dissolved in 400 mL of methanol by sonication for ten minutes, the vacuum pump was used to filter the mixture through Whatman No. 1. To obtain the final leaf methanol extract, the extract was next concentrated using a rotary evaporator at 40–50 °C under decreased pressure. The extract was kept at -8°C until it was needed.

2.2 Animal Purchase and Management

Thirty (30) mature male Wistar rats, each weighing 180–200g, were bought from a breeding facility in the Animal House of the University of Nigeria, Nsukka's Department of Pharmacology and Technology. Before being used in the experiment, they were given free access to clean water and standard livestock pellets (Guinea Feed Nigeria Limited) and kept in a well-ventilated iron cage with controlled conditions of 28ºC, 50% humidity, and 12-hour light/12-hour dark cycles.

2.3 Ethical Approval

Ethical approval (SMCCN/CREC/2024/004) was obtained and the study followed the guideline on animal research of Sancta Maria Catholic College of Nursing Sciences, Uzairue, Edo State

2.4 Procurement of Alloxan

Alloxan was purchased from Sogladen pharmaceutical store, Jattu, Etsako-west, Edo State, Nigeria.

2.5 Induction of Diabetes

Alloxan (1.5ml/kg) was orally given for 7 days to cause diabetes in the rats. After alloxan was administered on the first day, the rats were tested or checked daily for measurement of fasting blood sugar level, using Accu-check glucometer. Diabetic rats were certified by the fasting blood glucose level above 15mmol/L at the 7th day after alloxan given.

2.6 Experimental Design

Thirty (30) mature male Wistar rats of 180-200g were used for this study. They were divided at random into six (6) various groups of 5 rats each. Groups 1 severed as normal control; the rats were fed with normal saline and feed only, and were not exposed to alloxan and treatment. Alloxan were induced into group 2,3,4,5 and 6. Group 2 served as negative control; they were induced with alloxan but not given any treatment, just fed with normal saline and feed. Rats in groups 3, 4 and 5 were all exposed to alloxan and given extract at different doses respectively. The group 6 were the positive control group (drug group); the rats were given feed and treated with Vitamin E (50mg/kg, oral) (Table 1).

Table 1. *Experimental design*

Group	Description	Treatment
Group 1	Normal	Normal saline + feed
Group 2	Negative control	Alloxan 1.5ml + 0.5ml/kg of Normal saline
Group 3	Treatment group Low dose	Alloxan 1.5ml + 0.5ml/kg of extract
Group 4	Treatment group Medium dose	Alloxan 1.5ml + 1ml/kg of extract
Group 5	Treatment group High dose	Alloxan 1.5ml + 1.5ml/kg of extract
Group 6	Drug control	Alloxan 1.5ml + 1.5ml of standard drug

The rats were induced alloxan for a period of 7 days. After the confirmation of diabetes, extract administration follows for 14days. 24 hours after the last administration of extract, the animals were sacrificed using cervical dislocation methods.

2.7 Sacrifice and Collection of Specimens

At the end of the experiment, 24 hours following the final time extracts were administered the animals were dissected, the pancreas were cautiously harvested and prepared for histological examination.

2.8 Histological Studies

The rat's pancreas was carefully dissected out and processed through the stages of fixation in formalin. After fixation, they were dehydrated in ascending

grades of alcohol and embedded in molten paraffin wax. Coronal paraffin sections were cut and sections were mounted onto a gelatin-coated slides then stained using hematoxylin and eosin (H and E) stain. Thereafter, slides were viewed with digital light microscope. Hematoxylin is used to illustrate nuclear details in cells. It is one of the principal tissue stains used in histology. Haematoxylin stains cell nuclei a purplish blue, Eosin stains the extracellular matrix and cytoplasm pink, with other structures taking on different shades, and combination of these colours.

2.9 Statistical Data

The data collected were recorded at M + SED (standard error difference) and were analyzed using SPSS package version 23, ANOVA were used to compare the differences in the means of the various variables where significant, a post-hoc analysis using tukey test were carried out.

3. Results

3.1 Results of Mean Body Weight across the Groups

The mean weight of the Low dose extract was significantly ($p<0.05$, ANOVA) higher compared to the Alloxan only, Medium and high dose extract and standard drug groups. At Between days 9 and 12 remarkable increase in the weight of the Alloxan only group was observed

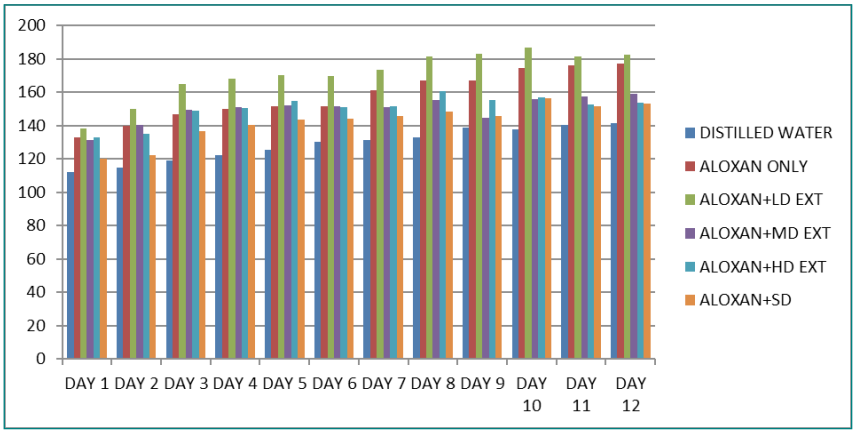

Figure 1. *Daily Weight: The mean weight in the Alloxan only group and treatment groups was significantly ($p<0.05$, ANOVA) higher compared to the distilled water group*

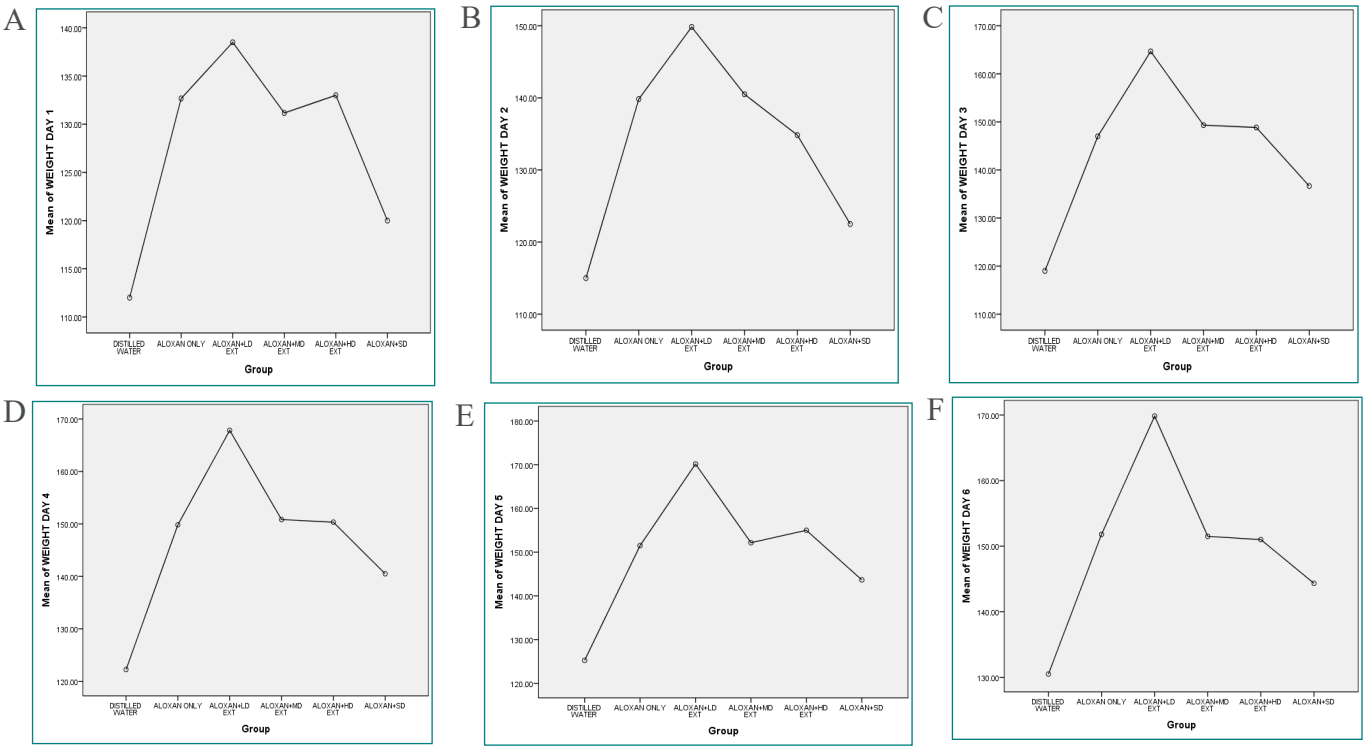

Figure 2. *Mean weights (A): Mean Weight on Day 1 after Induction; (B): Mean Weight on Day 2 after Induction; (C): Mean Weight on Day 3 after Induction; (D): Mean Weight on Day 4 after Induction; (E): Mean Weight on Day 5 after Induction; (F): Mean Weight on Day 6 after Induction;*

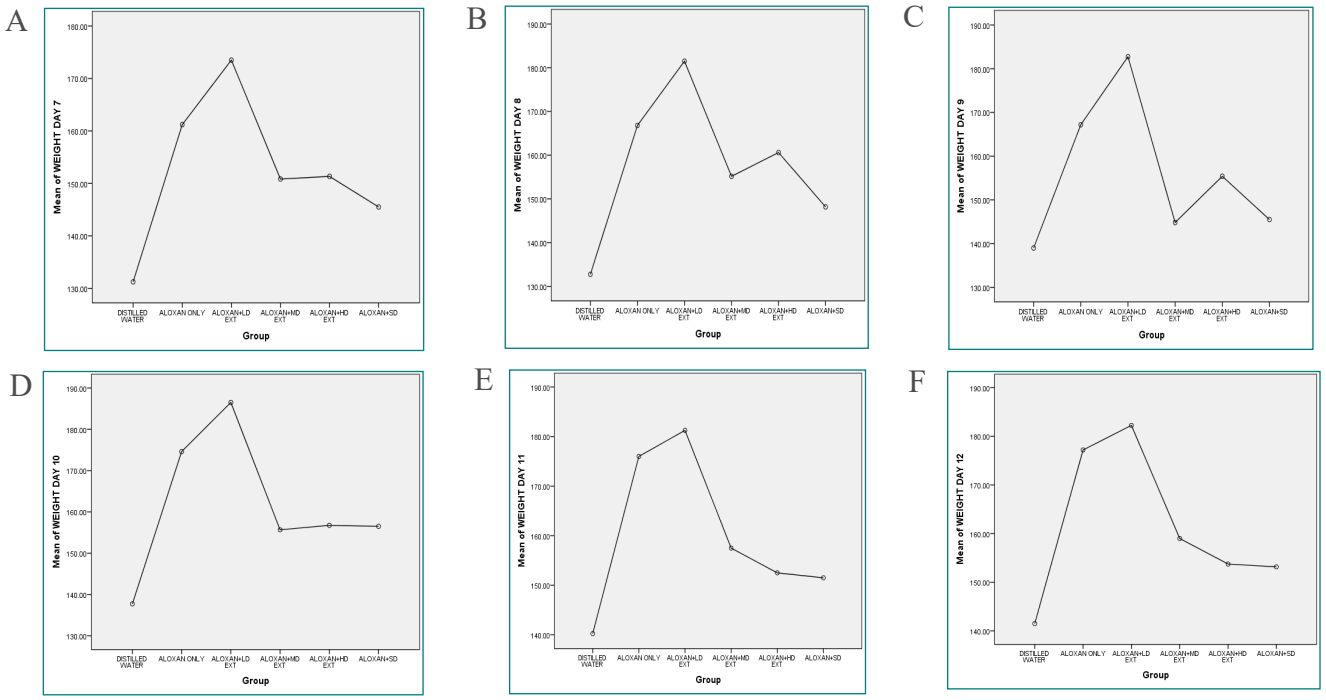

Figure 3. *Mean weights (A): Mean Weight on Day 7 after Induction; (B): Mean Weight on Day 8 after Induction; (C): Mean Weight on Day 9 after Induction; (D): Mean Weight on Day 10 after Induction; (E): Mean Weight on Day 11 after Induction; (F): Mean Weight on Day 12 after Induction*

In Figure 2 (A), the mean weight in the Alloxan only group and treatment groups were notably ($p<0.05$, ANOVA) higher in contrast to the distilled water group. The mean weight of the minimal dose extract was significantly ($p<0.05$, ANOVA) higher in contrast to the Alloxan only, Medium and high dose extract and standard drug groups. The mean weight in the Standard drug group was significantly ($p<0.05$, ANOVA) lower compared to the treatment groups.

In Figure 2 (B), the mean weight in the Alloxan only group and treatment groups were substantially ($p<0.05$, ANOVA) higher in contrast to the distilled water group. The mean weight of the Low dose extract was notably ($p<0.05$, ANOVA) higher as opposed to the Alloxan only, Medium and high dose extract and standard drug groups. The mean weight in the Standard drug group was substantially ($p<0.05$, ANOVA) lower in comparative to the treatment groups

In Figure 2 (C), the mean weight in the Alloxan only group and treatment groups were substantially ($p<0.05$, ANOVA) higher as opposed to the distilled water group. The mean weight of the Low dose extract was much ($p<0.05$, ANOVA) higher as opposed to the Alloxan only, Medium and high dose extract and standard drug groups. The mean weight in the Standard drug group was significantly ($p<0.05$, ANOVA) lower compared to the treatment groups.

In Figure 2 (D), the mean weight in the Alloxan only group and treatment groups were significantly ($p<0.05$, ANOVA) higher compared to the distilled water group. The mean weight of the Low dose extract was substantially ($p<0.05$, ANOVA) higher compared to the Alloxan only, Medium and high dose extract and standard drug groups. The mean weight in the Standard drug group was significantly ($p<0.05$, ANOVA) lower in contrast to the treatment groups.

In Figure 2 E, the mean weight in the Alloxan only group and treatment groups were significantly ($p<0.05$, ANOVA) higher compared to the distilled water group. The mean weight of the Low dose extract was significantly ($p<0.05$, ANOVA) higher compared to the Alloxan only, Medium and high dose extract and standard drug groups. The mean weight in the Standard drug group was significantly ($p<0.05$, ANOVA) lower compared to the treatment groups.

In Figure 2 (F), the mean weight in the Alloxan only group and treatment groups were significantly ($p<0.05$, ANOVA) higher compared to the distilled water group. The mean weight of the Low dose extract was significantly ($p<0.05$, ANOVA) higher compared to the Alloxan only, Medium and high dose extract and standard drug groups. The mean weight in the Standard drug group was significantly ($p<0.05$, ANOVA) lower compared to the treatment groups

In Figure 3 (A), the mean weight in the Alloxan only group and treatment groups were significantly ($p<0.05$, ANOVA) higher compared to the distilled water group. The mean weight of the Low dose

extract was significantly ($p<0.05$, ANOVA) higher compared to the Alloxan only, Medium and high dose extract and standard drug groups. The mean weight in the Standard drug group was significantly ($p<0.05$, ANOVA) lower compared to the treatment groups.

In Figure 3(B), the mean weight in the Alloxan only group and treatment groups were significantly ($p<0.05$, ANOVA) higher compared to the distilled water group. The mean weight of the Low dose extract was significantly ($p<0.05$, ANOVA) higher compared to the Alloxan only, Medium and high dose extract and standard drug groups. The mean weight in the Standard drug group was significantly ($p<0.05$, ANOVA) lower compared to the treatment groups.

In Figure 3 (C), the mean weight in the Alloxan only group and treatment groups were significantly ($p<0.05$, ANOVA) higher compared to the distilled water group. The mean weight of the Low dose extract was significantly ($p<0.05$, ANOVA) higher compared to the Alloxan only, Medium and high dose extract and standard drug groups.

In Figure 3 (D), the mean weight in the Alloxan only group and treatment groups were significantly ($p<0.05$, ANOVA) higher compared to the distilled water group. The mean weight of the Low dose extract was significantly ($p<0.05$, ANOVA) higher compared to the Alloxan only, Medium and high dose extract and standard drug groups.

In Figure 3 (E), the mean weight in the Alloxan only group and treatment groups were significantly ($p<0.05$, ANOVA) higher compared to the distilled water group. The mean weight of the Low dose extract was significantly ($p<0.05$, ANOVA) higher compared to the Alloxan only, Medium and high dose extract and standard drug groups.

In Figure 3 (F), the mean weight in the Alloxan only group and treatment groups were significantly ($p<0.05$, ANOVA) higher compared to the distilled water group. The mean weight of the Low dose extract was significantly ($p<0.05$, ANOVA) higher compared to the Alloxan only, Medium and high dose extract and standard drug groups.

3.2 Histological Analysis Result

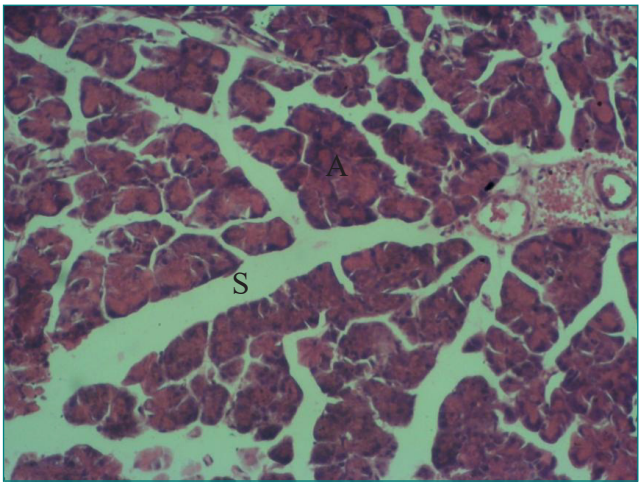

Plate 1. *Normal control group: received normal saline and feed: Group A: Photomicrograph of the pancrease showing normal acini tissue (a) with septa (s). General tissue appears normal. H & E. X200*

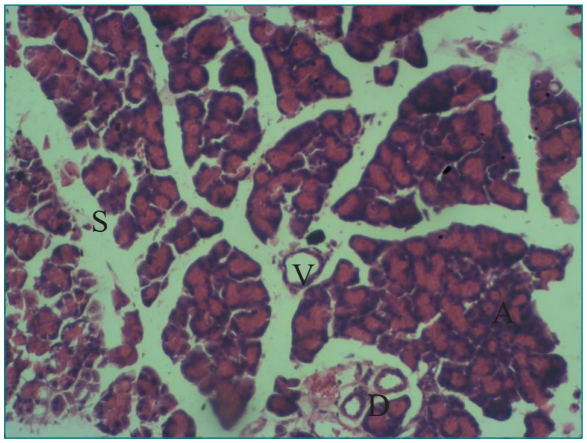

Plate 2. *Negative control group; induced with alloxan and received normal saline. Group B: Photomicrograph of the pancrease showing normal acini tissue (a) with septa (s). General tissue shows a duct(d) and vein(v). H & E. X200*

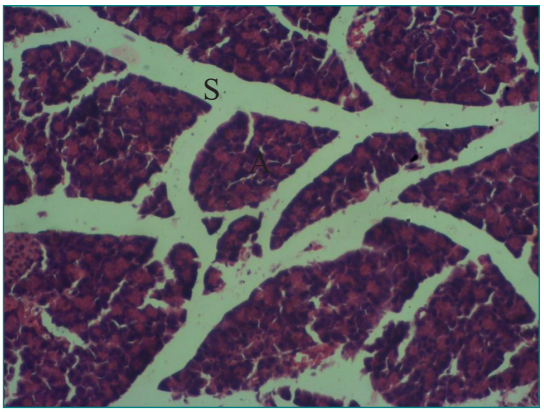

Plate 3. *Low dose treatment group; induced with alloxan and treated with Vernonia amygdalina extract. Group C: Photomicrograph of the pancrease showing normal acini tissue (a) with septa (s). General tissue appears normal. H & E. X200*

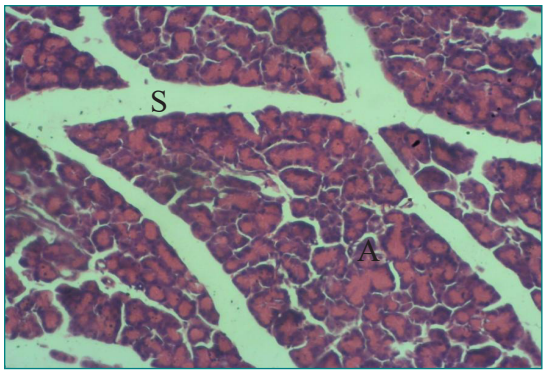

Plate 4. *Medium dose treatment group; induced with alloxan and treated with Vernonia amygdalina extract. Group D: Photomicrograph of the pancrease showing normal acini tissue (a) with septa (s). General tissue appears normal. H & E. X200*

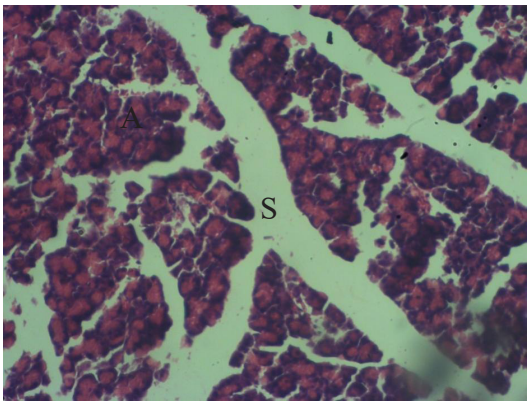

Plate 5. *High dose treatment group; induced with alloxan and treated with Vernonia amygdalina extract. Group E: Photomicrograph of the pancrease showing normal acini tissue (a) with septa (s). General tissue appears normal. H & E. X200*

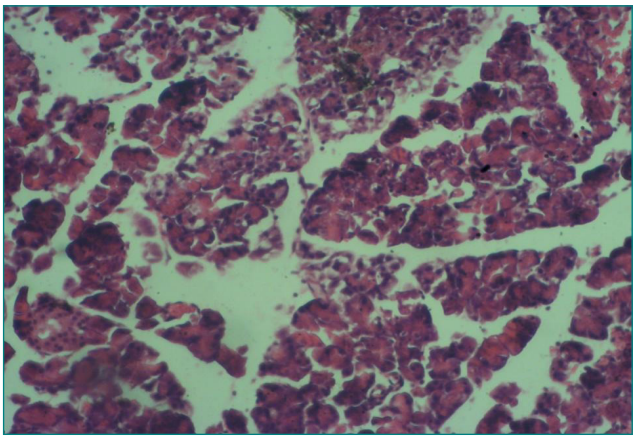

Plate 6. *Standard drug group; induced with alloxan and treated with vitamin E. Group F1: Photomicrograph of the pancrease showing mild acini derangement. H & E.X200*

4. Discussion

Alloxan induction in rats is a tried-and-true technique for creating type 1 or insulin-dependent diabetes. Therefore, the purpose of this study is to investigate the preventive diabetic properties of *Vernonia amygdalina* leaf extracts on the microstructure of the pancreas of adult wistar rats following alloxan-induced diabetes.

Numerous studies have demonstrated that when animals develop diabetes due to alloxan, their weight loss often significantly slows down. [21, 22] Body weight decreases as a result of increased protein breakdown brought on by the inability to get carbohydrates for energy synthesis. This assertion was validated by our research (Figure 1). The body weight returned to normal in the group that had *Vernonia amygdalina* treatment. Decreased protein catabolism might be the cause of this. Maintaining normal serum blood glucose levels is a key component of diabetes treatment, as hyperglycemia is one of the major symptoms of the disease. Serum glucose levels in the alloxan-diabetic rats in this research significantly decreased after receiving *Vernonia amygdalina* treatment. The process by which *Vernonia amygdalina* reversed the decreased in body weight as seen in low dose *Vernonia amygdalina group* (Figure 3) level is unknown when compared to distilled water, alloxan only, medium dose, high dose and standard drug groups. Bihonegn *et al.*[23], in their studies revealed that *Vernonia amygdalina* are anticipated to prevent decrease in body weight and *Vernonia amygdalina* leaves substantially inhibited weight reduction in rats. However, *Vernonia amygdalina* has a positive impact on diabetes since it has the ability to revitalize pancreatic cells over an extended period of therapy.[24] Treatment with 0.5ml extract of *Vernonia amygdalina* demonstrated the potency of *Vernonia amygdalina* which is agrees with the findings Onyibe *et al.*[24]

Numerous studies have demonstrated that persistent hyperglycemia results in advanced metabolic and morphological changes in a number of tissues, including the kidney, brain, pancreas, and other organs.[25-29] It has been proposed that one of the main mechanisms of diabetes is the overproduction of ROS in these tissues, which raises lipid peroxidation and lowers antioxidant enzyme activity.[30]

Histological observations gotten from this study showed that 1.5ml/kg of Alloxan caused mild acini metaplasia (Plate 2) and focal area of acini aplasia compared to the control group whose pancreas microstructure appeared normal (Plate 1). Decrease in β-cell mass is critical in the pathogenesis of diabetes mellitus. According to morphological data, the Alloxan groups' pancreatic islets were significantly smaller than those of the normal control group, which is consistent with findings that have been previously reported.[31, 32]

However, *Vernonia amygdalina* extract therapy was able to decrease islet cells loss likewise metaplasia and aplasia of acini and showed significant increase in a dose dependent manner. These results indicate that giving *Vernonia amygdalina* to diabetic rats has a positive effect on the regeneration of β-cells in damaged pancreas, as shown in Plates 3,4 and 5 compared with Plates 1,2 and 6. It is ascertained that β-cells can regenerate either by neogenesis from stem cells and progenitor cells inside or outside the islets, or by replicating pre-existing β-cells.[33] The extract from Vernonia amygdalina having a regenerative impact on β-cells might be because of its antioxidant properties. Recent research has shown that oxidative stress and β-cell regeneration are closely related biological processes. This is evident not only from the fact that they coexist in a variety of physiological and pathological settings, but also from the significant negative effects that oxidative stress has on β-cell regeneration, both directly and indirectly.[30] Additionally, a number of studies have demonstrated the positive benefits of antioxidant supplementation on the regeneration of β-cells.[24, 34]

5. Conclusion

The present study reveals that *Vernonia amygdalina* extract has strong antidiabetic effect in rats with diabetes caused by alloxan. The results of this study show that Vernonia amygdalina extract protects against damage to the pancreatic islets, can regenerate β-cells, and, in a dosage-dependent way, improves weight loss in rats with diabetes caused by alloxan. These benefits are likely due to the extract's antioxidant properties.

Conflict of Interest

The authors declare no conflict of interest.

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