

Possible Revival of Atrophied Islet Cells of the Pancreas by *Vernonia amygdalina* in Alloxan Induced Diabetic Rats.

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ABSTRACT

The Possible Revival of atrophied islet cells of the pancreas by *Vernonia amygdalina* in alloxan-induced diabetic rats was evaluated. Twenty rats were divided into five groups (1, 2, 3, 4 and 5) of four rats each. Group 1 rats were given only feed and distilled water (normal control) throughout the period of the experiment. Group 3, 4 and 5 rats were pretreated with 250mg/kg body weight of the extract for 7, 14 and 21 days respectively. Diabetes was induced in rats in group 2, 3, 4 and 5 with 150mg/kg of body weight of alloxan monohydrate. Group 2 rats were used as the experimental control. Fasting blood glucose of rats in all the groups were measured before and 72 hours after induction of diabetes. The rats were sacrificed after 72 hours and the pancreas was histopathologically analysed. The result showed a significantly high blood glucose level in group 2 rats indicating the diabetic state. The blood glucose level of rats in group 3 and 5 reduced significantly ($p < 0.05$) when compared with the value of group 2 rats but not significantly different from group 1 value. Group 4 showed a significantly ($p < 0.05$) high blood glucose level when compared with the base-line. The histopathology revealed atrophied islet of Langerhans in group 2 rats. Group 3 and 5, showed reviving islet cells and group 4 showed increased lymphoid follicles and neutrophils. The results suggest that the aqueous extract of *V. amygdalina* has a protective effect against alloxan induced pancreatic damage and potential to revive damaged islet cells.

INTRODUCTION

Diabetes mellitus is a disease condition characterized by high blood glucose levels caused by an absolute or relative deficiency of insulin. The causes of diabetes mellitus include poor diet, obesity, environmental factors, hereditary factors and drug usage. Symptoms are polyuria, polyphagia, polydipsia, loss of water and loss of memory. Complications of diabetes mellitus include damage to kidneys, impaired vision, hardening and blockage of blood vessels, infections and impotence (Famuyiwa, *et al*, 1998). Alloxan, a chemical used to induce

experimental diabetes mellitus, causes the beta cells of the islets of langerhans to swell and finally degenerate. Alloxan diabetic rats have been reported to have increased vascular permeability, nerve fiber loss. The increasing incidence of diabetes is alarming and the modes of treatment are still inappropriate among the traditional healers, despite the wide range of herbal products at our disposal. Medicinal plants have always been considered a healthy source of life for all people. Therapeutic properties of medicinal plants are very useful in healing various diseases and the advantage of medicinal plants is being 100% natural. Many of the edible vegetables in our environment are known to possess biological activities that are beneficial in preventing or managing diseases. For example popular vegetables such as *Vernonia amygdalina*,

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Vernonia amygdalina, also commonly called bitter leaf because of its bitter taste, is a vegetable commonly used regularly in many West Africa sub-regions, particularly Nigeria. The leaves may be consumed either as a vegetable (Macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illness. It belongs to the plant family compositae.

The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides. Hamowia and Saffaf, 1994 reported that it exhibited cytotoxicity and antitumour properties in animal models. *Vernonia amygdalina* is used traditionally to treat kidney problems and hiccups and as stomachic, purgatives, anthelmintics, anti-asthmatic carminatives and antitumor agents (Hamowia and Saffaf, 1994). Components such as vernodalin and vernoanydalin found in the extracts of *V. amygdalina* del have been found to inhibit invitro growth of cell derived from human carcinoma of the nasopharynx (Kupcham, *et al.*, 1969). Philipson *et al.*, 1993 reported the antiplasmodial effects of sesquiterpene and steroidal constituents of *V. amygdalina* and that they are also effective against plasmodium falciparum in vitro. Teas containing bitter leaf are also used throughout West Africa for the management of diabetes and other metabolic diseases associated with the liver. The plant has acquired special relevance lately, having been proved in human medicine to possess potent anti-malarial and antihelmintic properties (Abosi and Raseoka, 2003) as well as anti-tumorigenic properties (Izevbogie, 2003).

Pharmacological studies have also shown that the leaf extract has both hypoglycaemic and hypolipidaemic properties in experimental animals and so could be used in managing diabetes mellitus (Akah and Okafor, 1992). Uhegbu and Ogbuehi (2004) reported the anti-diabetic effects of aqueous extracts of leaves of *Vernonia amygdalina*. Extracts of the plant have also been used traditionally in Nigeria and other cultures as a tonic in the treatment of sexually transmitted diseases, feverish condition, cough, constipation, and hypertension (Kambizi and Afolayan, 2001). Oral administration of the aqueous leaf extract of the plant was found to relieve pain and lower body temperature. Considering these enormous anti-diabetic properties of *Vernonia amygdalina*, it is necessary to investigate its possible revival of alloxan-induced atrophied islet cells in experimental animals. This will be done by carrying out histopathological studies (microscopic examination of tissues in order to study the manifestation of diseases) of the islet cells of the pancreas.

MATERIALS AND METHODS

Plant Materials and Extract Preparation

Fresh *Vernonia amygdalina* leaves were collected from a local garden within Igbinedion University premises and authenticated at the Botany Department, Igbinedion University, Okada. They were air-dried and ground into fine powder. The powder was soaked in distilled water for 24 hours, filtered using a sintered funnel and the extract concentrated using a rotary evaporator and weighed.

Experimental Animals

Adult albino rats of the Wistar strain, weighing between 140 – 210g were obtained from the animal house of Igbinedion University, Okada. They were housed in clean standard disinfected cages with a 12 hour light/dark cycle and 50-60% relative humidity, at a temperature of about 30°C. The animals were allowed free access to feed (guinea grower's mash) and tap water, and were treated according to the International guidelines for the care and use of laboratory animals. They were allowed to acclimatize to the new environment for a period of two weeks, after which they were randomized into five groups (groups 1, 2, 3, 4 and 5) of four animals each and weighed.

Induction of Diabetes

Diabetes was induced by administration of alloxan (Alloxan monohydrate obtained from BHD Chemicals, Poole, England.), intra-peritoneally, at a dose of 150mg/kg body weight.

Treatment

The extract was administered orally at a dose of 250mg/kg body weight.

Experimental Design

The experimental animals were randomized into five groups (groups 1, 2, 3, 4 and 5) of four animals each and treated as follows;

Group 1: Given distilled water only throughout the experimental period and served as normal control.

Group 2: Induced with alloxan only and not pre-treated with the extract. This served as Diabetic control.

Group 3: Pre-treated with the extract and then induced with alloxan on the 7th day.

Group 4: Pre-treated with the extract and then induced with alloxan on the 14th day.

Group 5: Pre-treated with the extract and then induced with alloxan on the 21st day.

The animals were administered with the extract daily. Alloxan was administered on the specified days after which the experimental animals were fasted overnight prior to blood glucose determination. Fasting blood glucose of rats in all the groups were measured before and 72 hours after induction of diabetes. The rats were sacrificed after 72 hours and their pancreas harvested for histopathological studies. Blood glucose level was determined using the glucometer.

RESULTS AND DISCUSSION

The effect of *Vernonia amygdalina* on fasting blood glucose of diabetic rats is shown in table 1. The result showed a significant ($p < 0.05$) increase in group 2 which were not pre treated with the aqueous extract when compared to the normal control (Group 1). Group 3 showed a significantly ($p < 0.05$) lower fasting blood glucose when compared to diabetic group (Group 2) that were not pretreated (experimental control). Group 4 showed no

significant difference from group 2 but increased significantly when compared with Group1 (baseline). The result obtained from group 5 is similar to that of group 3 indicating the hypoglycemic effect of the extract.

The pancreas was harvested to show the effect of the alloxan and the extract and the following were observed.

Table. 1: Effects of aqueous leaf extract of *Vernonia amygdalina* on fasting blood glucose (mg/dl) of experimental animals.

GROUP	DAY		
	7	14	21
1	113.25 ± 4.71	113.25 ± 5.32	113.50 ± 6.66
2	366.33 ± 9.07*	-	-
3	116.67 ± 11.93**	-	-
4	-	363.00 ± 10.00	-
5	-	-	123.00 ± 5.66**

Values are expressed as MEAN ± S.E.M, n = 4, *p < 0.05 (Group 2 significantly higher than Group 1), **p < 0.05 (Group 3 & 5 significantly lower than Group 2).

Group. 1:Control

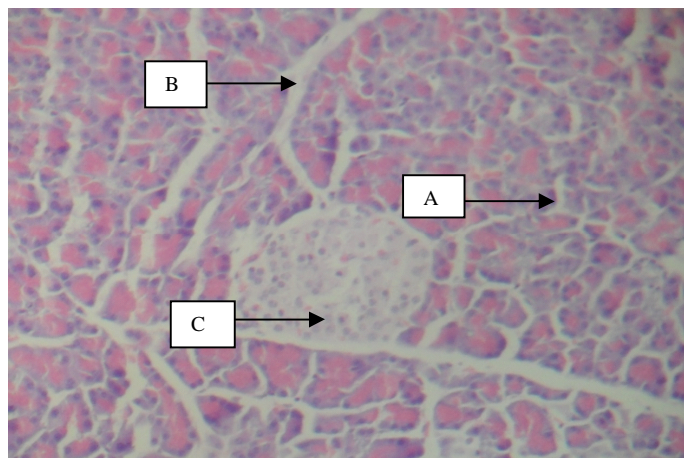


Fig . 1: Normal Rat Pancreas showing exocrine glands A, interlobar septa B and Islets of Langerhans C (H & E x 10).

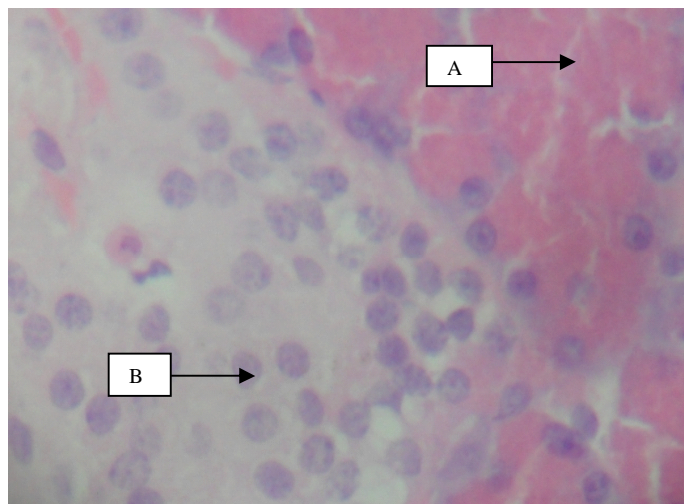


Fig. 2: Higher Magnification of the structure above showing islets A, and exocrine glands B (H & E x 40)

Group . 2: Given Alloxan Only on Day 7.

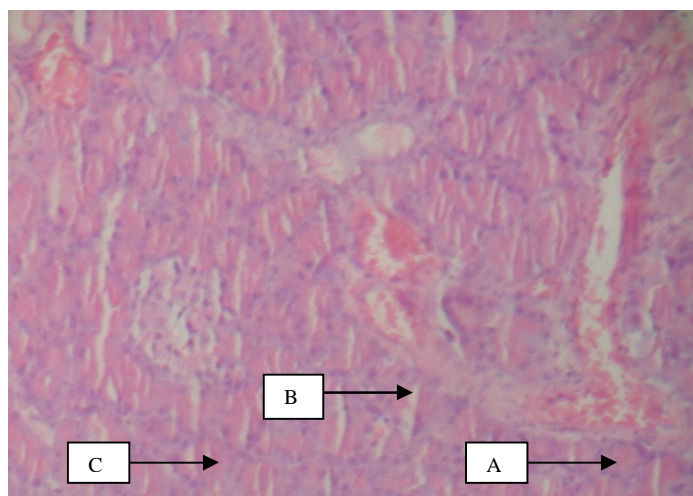


Fig. 3: Rat Pancreas given alloxan after 7 days showing atrophied islets A, moderate vascular congestion B and hypertrophy C (H & E x 10)

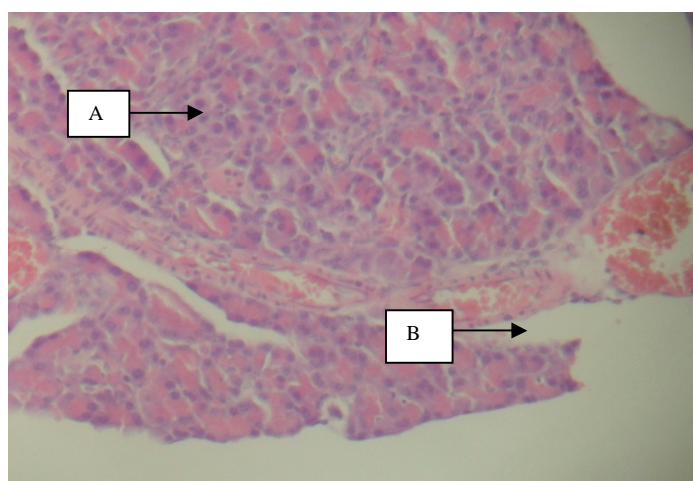


Fig . 4: Rat Pancreas treated with alloxan after 7 days showing atrophied islets A and severe vascular congestion B (H & E x 10).

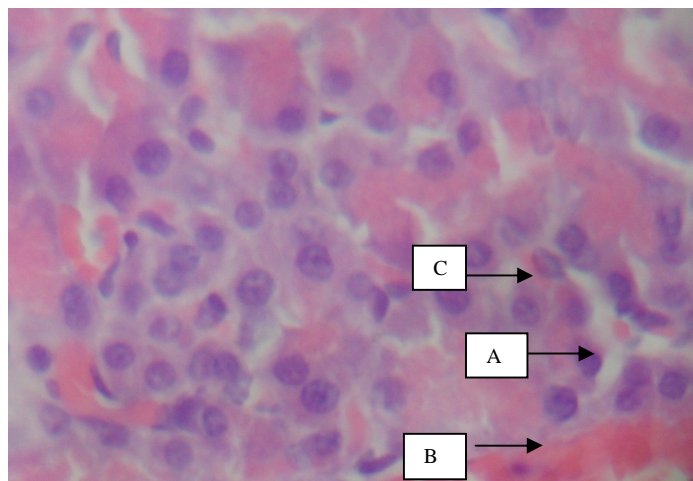


Fig . 5: Higher magnification of the structure above showing degenerating islets A, severe congestion B and haemorrhage C (H & E x 40) Sections showed atrophic islets of langerhans, moderate interlobar vascular congestion and mild haemorrhage. Summary: There is destruction of the endocrine glands of the pancreas

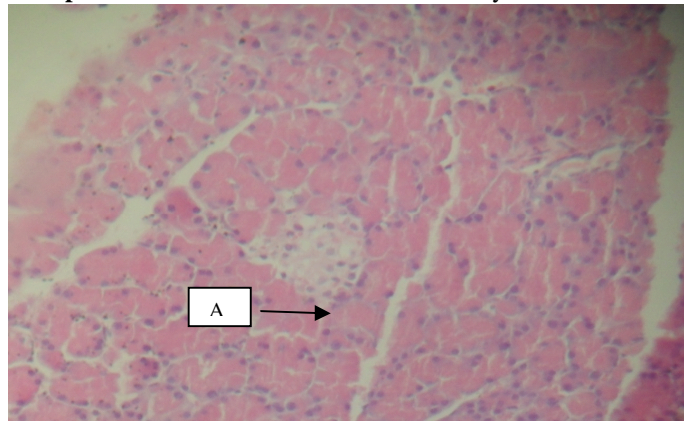
Group. 3: Given Extract Then Alloxan On Day 7.

Fig. 6: Rat Pancreas given *Vernonia amygdalina* then alloxan for 7 days showing mildly regenerating islets A (H & E x 10)

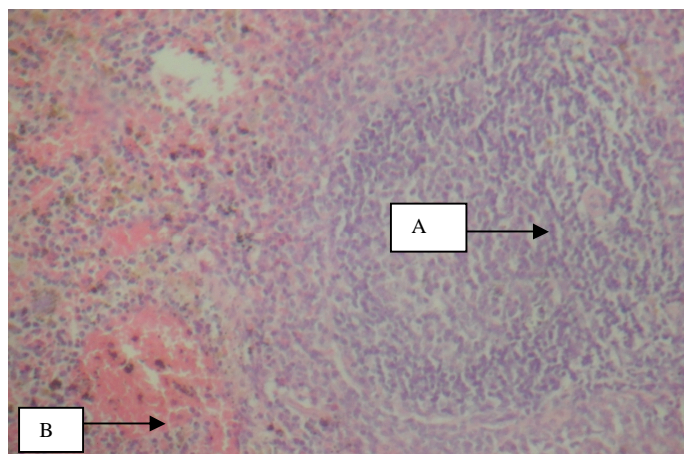


Fig. 7: Another field of the same pancreas as above showing floridly activated lymphoid follicle A and moderate congestion B (H & E 10)

Sections showed recovering but small islets, moderate vascular congestion and florid activation of peripancreatic lymphoid follicles.

Summary: There is mild recovery of the islets, but marked activation of local immune system

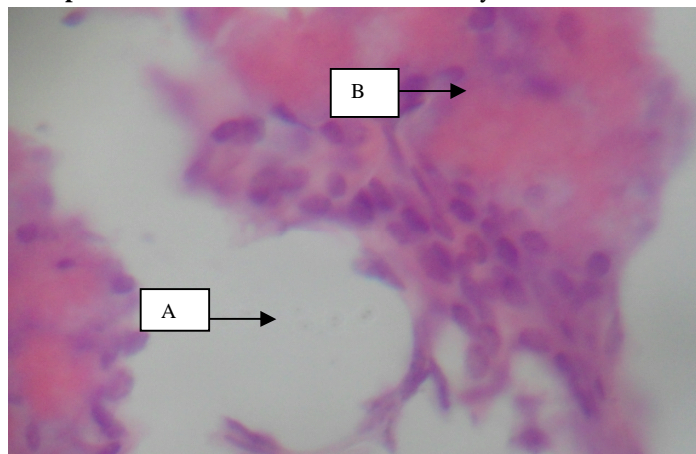
Group. 4: Given Extract Then Alloxan On Day 14.

Fig 8: Rat pancreas given *Vernonia amygdalina* then alloxan after 14 days, showing moderate vascular congestion A and mild infiltrates of lymphocytes B (H & E x 40). Sections showed mild islet recovery, mild vascular congestion and mild peri-islet infiltrates of lymphocytes. SUMMARY: There is still mild recovery of the islets as well as mild inflammation

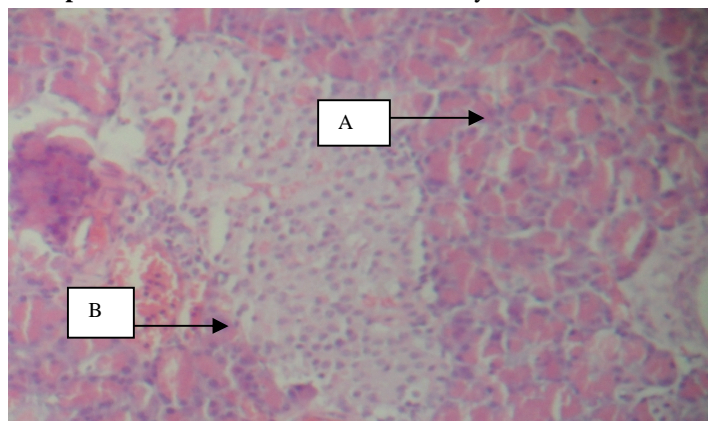
Group 5: Given Extract Then Alloxan On Day 21.

Fig 9: Rat pancreas given *Vernonia amygdalina* then alloxan after 21 days showing moderately regenerating islets A and mild vascular congestion B (H & E x 10).

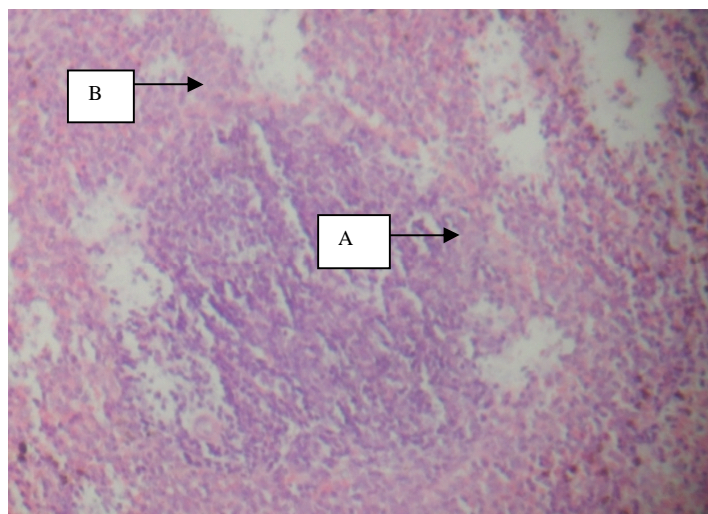


Fig. 10: Another field of the same pancreas as above showing mildly activated lymphoid follicle A and mild congestion B (H & E 10). Sections showed moderate recovery of islets and mildly activated lymphoid follicles. SUMMARY: There is moderate recovery of the islets and mildly activated local immune system.

Medicinal plants have been in use by man since time immemorial for prevention, management and treatment of ailments. The current study investigated the possible revival effect of *V. amygdalina* in alloxan induced pancreatic tissue damage. *V. amygdalina*, an edible plant, commonly called bitter leaf has been used in folklore medicine for treatment of diabetes and several other disorders. There has been evidence for the use of the plant extract in lowering blood glucose level (Yeap *et al.*, 2010).

The results obtained from this study as presented in table 1 reveal the effectiveness of the plant extract at preventing pancreatic damage that may result in increased blood glucose. The experimental animals were made diabetic using alloxan monohydrate. Alloxan monohydrate has been reported to induce tissue damage in rats (Omamoto *et al.*, 1981). The mechanism of action of this chemical is via free radical induced tissue damage, causing oxidative stress in the tissue involved, specifically the pancreas.

The pancreas releases insulin under high glucose tension to increase its uptake and utilization by cells. Damaged islets cells of the pancreas, especially when the β cells are affected, could result in impairment of insulin release and as such, affect blood glucose level.

In Table 1, rats in the experimental control group (Group 2) showed a significantly ($p < 0.05$) high blood glucose level following alloxan induction revealing the toxicity of the chemicals. Group 3, showed the protective effect of the extract on diabetic rats. *V. amygdalina* has been characterized to contain antioxidants including, flavonoids, phenolics, alkaloids and some glycosides (Youdim *et al.*, 2007). There is a positive correlation between flavonoids component of a plant extract and its ability to revive free radicals damaged cells. Thus, this components of the extract, is possibly what accounts for the diabetic preventive property of the aqueous extract of *V. amygdalina*. However the glucose level of rats in group 4 increased significantly when compared with the baseline, showing that the extract has no effect on rats in this group. Accumulation of the extract in the rat's gastro intestinal tract could hinder its metabolism, thereby prolonging the insulin secretion time, thus showing no effect on rats on this day. This is justified by the ability of the extract to reduce the effect of alloxan on Day 21 as observed in group 5.

Further tissue (pancreas) examination following histopathology showed that the pancreas of this group reduced in size which could alter the release of insulin and thus, hinder glucose uptake. Group 3 and 5 showed the reviving of islet cells which increases insulin release. They also showed increase in lymphocytes as a means of self-regulation and protection. Inflammation or necrosis of cells could result into increase in lymphoid follicles and neutrophils in response to tissue damage. The incidence of diabetes surged over the years with no defined measure of treatments, thus, a need for prevention, to revive and increase the integrity of pancreatic cells. The aqueous extract of *v. amygdalina* as depicted from the result has the ability to maintain the integrity of pancreatic cells.

CONCLUSION

The aqueous extract of *Vernonia amygdalina* showed protective property against alloxan induced diabetes. However, this study gives room for further toxicity and mechanistic study of the extract at protecting and reviving the atrophied islet cells. This will increase the acceptability and control of the use of the plant extracts. This will also help Nigerians to spend less and gain more in the management of diabetes mellitus.

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