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Effect of *Vernonia amygdalina* on the Serum Amylin and Insulin Levels of Alloxan-Induced Diabetic Rats

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Abstract:
Vernonia amygdalina extracts have been reported to possess hypoglycemic properties but the mechanism is not yet known. This study investigated the effect of aqueous and ethanolic extracts of Vernonia amygdalina on the amylin and insulin levels of diabetic rats. The rats were induced with 5% alloxan solution at a single subcutaneous dose of 150mg/kg body weight. The glucose level of the diabetic rats significantly increased ($p < 0.05$) to the range of 260 – 275mg/dl following alloxan injection. Treatment of the diabetic rats with aqueous and ethanolic extracts of Vernonia amygdalina resulted in significant reduction ($p < 0.05$) of the glucose levels. There were statistically significant ($p < 0.05$) increase in the amylin and insulin levels of diabetic rats treated with both extracts as compared to the diabetic untreated rats. The results showed that Vernonia amygdalina extracts possess hypoglycemic effect by enhancing the secretion of amylin and insulin from the pancreas.

Keywords: *Amylin, Insulin, Vernonia amygdalina*

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by increased blood glucose levels (hyperglycemia) due to lack of insulin production or deficiencies in insulin action and secretion(Abdulazeez *et al.,* 2013). It is a chronic disorder that can lead to severe cardiovascular, retinal, neurological and renal complications (Khawaja *et al.,* 2004; Shera *et al.,* 2004). Amylin (also known as islet amyloid polypeptide or IAPP) is a37-amino acid, peptide hormone that is co-localised, co-secreted and co-stored with insulin by pancreatic β-cells (Cooper*et al.,* 1987; Westermark *et al.,*1986). Amylin is actually secreted together with insulin in a 20 to 1 molar ratio of insulin to amylin (Lartin, 2006).Studies have suggested that amylin plays a crucial role in glucose homeostasis by suppressing the release of glucagon from pancreatic α-cells, and hence prevent the release of glucose from the liver, decreases gastric emptying, and stimulates the satiety center in the brain (Zhang *et al*, 2016; Akesson *et al.,* 2003; Cooper, 1995).However, amylin aggregation may induce β-cell damage and apoptosis resulting in β-cell dysfunction and absolute loss of β-cell mass (Kodali and Wetzel, 2007; Bai *et al*., 1999).

Studies have shown that diabetes could only be managed by the use of hypoglycemic agents, most of which are expensive with undesirable side effects and contraindications or both (Jouhari *et al.,* 2000; Tunbridge and Home, 1991). As such, the World Health Organisation (WHO) recommended the need for the development and evaluation of better, safer and affordable pharmacological agents for improving insulin secretion, enhancing insulin sensitivity, preventing beta cell destruction and promoting beta cell regeneration or repair (WHO, 1994; WHO, 1985). This has stimulated interest on the research of anti-diabetic medicinal plants.

*Vernonia amygdalina*Del is a common shrub or small tree that grows throughout tropical Africa. It is commonly referred to as bitter leaf because of its abundant bitter part and the leaves are used in Nigeria for both nutritional and therapeutic purposes. It is used as vegetables in soups and in the treatment of malaria, diabetes mellitus, venereal disease, wounds, hepatitis and Cancer (Erasto *et al*., 2007; Kambizi and Afolayan 2001). Fresh aqueous extract of the leaves has been found to contain alkaloids, saponins, tannins, flavonoids and proteins (Akah and Okafor, 1992), as well as vitamins and minerals (Fafunso and Basir, 1977).

Extracts from the leaves have been reported to possess hypoglycemic and hypolipidemic properties, and have been found useful in the ethnotherapy of diabetes (Nwajo, 2005; Uhegbu and Ogbuehi, 2004; Akah and Okafor, 1992). Studies also revealed the plant extracts protect the kidneys and livers of alloxan-induced diabetic rats against complications (Atangwho *et al*., 2007; Mohammed *et al*., 2007; Nimenibo-Uadia, 2003).However, the mechanism by which it does these has not been established. As a result, this study aims to investigate the effect of *V. amygdalina* on the release of amylin and insulin in diabetic treated and untreated rats.

2. Materials and Methods

2.1. Plant Material and Extract Preparations (Abdulazeez et al., 2013)

V. amygdalina was purchased from Sokoto market and identified at the Botany Department of Usmanu Danfodiyo University, Sokoto. The fresh leaves were air dried and grinded into fine powder. 100g of the powdered leaves were soaked separately in 500ml of distilled water and ethanol for 24hr (1:5w/v). The extracts were sieved using a muslin cloth and then filtered with a Whatman's filter paper. All extracts were concentrated using a water bath at 40^{o}C and stored at 4°C until needed.

2.2. Experimental Animals

Adult albino rats (Wistar strains) of both sexes were obtained from the animal house of Biochemistry Department, Ahmadu Bello University, Zaria. The animals were allowed to acclamatise for 2 weeks and fed with standard commercial diet (Vital feed, Jos) and distilled water ad-libitum before the commencement of the experiment. The study was conducted with strict adherence to ethical procedure on the use of animals for experiments.

2.3. Induction of Diabetes Mellitus

Diabetes was induced by the subcutaneous injection of a single dose of alloxan monohydrate at 150mg/kg body weight. The rats with blood glucose level greater than 150mg/dl, two days post-induction (48-hour), were considered diabetic and were used for the experiment.

2.4. Experimental Groups

Experimental rats were divided into four groups of five rats each and the study was carried out for 4 weeks.

Group 1 – Normal control (distilled water)
Group 2 – Diabetic untreated rats
Group 3 – Diabetic rats treated with aqueous extract of *V. amygdalina*
Group 4 – Diabetic rats treated with ethanolic extract of *V. amygdalina*

2.5. Extracts Administration:

Each diabetic treated rat received 80mg/kg body weight of *V. amygdalina*, as previously determined as the optimum antidiabetic dose,for 2 weeks by oral route daily (Akah and Okafor, 1992).

2.6. Biochemical Analyses

The fasting serum glucose concentration was estimated by glucose oxidase method (Barham and Trinder, 1969). The determination of insulin was based on immunoenzymatic method of Kahn and Rosenthal (1979). Amylin determination was based on competitive ELISA method (Elabscience, USA).

2.7. Statistical Analysis

Data were expressed as means $\pm$ SEM. Statistical analysis was performed using Graphpad Instat software version 3.0 (USA). The means within a group was compared using student t-test, while Analysis of Variance (ANOVA) was used with Dunnett's posttest for comparisons of more than 2 groups.

3. Results

The effects of aqueous and ethanolic extracts of *Vernonia amygdalina* on the body weight of diabetic treated and untreated rats are presented in Table 1.The result showed the body weightof the rats significantly decreased ($p<0.05$) following alloxan injection but improved after the administration of the plant extracts.

	Day 1	Day 4	Day 18
Group 1	125.0 ± 16.79^{a}	134.4 ± 21.31^{a}	147.0 ± 12.21^{b}
Group 2	142.4 ± 6.07^{a}	131.8 ± 6.30^{a}	120.8 ± 6.58^{b}
Group 3	139.2 ± 6.60	133.2 ± 3.01	144.0 ± 11.98
Group 4	144.0 ± 7.98	141.5 ± 10.68	142.7 ± 21.53

Table 1: Effects of Vernonia amygdalina extracts on the body weight (g) of diabetic treated and untreated rats

Values are mean ± SEM. Values with different superscript on the same row are significantly different ($p< 0.05$). Day 1- Before alloxan injection, Day 4- Four days after alloxan injection, Day 18- After treatment.

The effects of aqueous and ethanolic extracts of *Vernonia amygdalina* on the serum glucose level of diabetic treated and untreated rats are presented in Table 2. There was a statistically significant increase ($p < 0.05$) in the glucose levels of the diabetic rats (groups 2 - 4) following the injection of alloxan as compared to the normal control (group 1). A significant decrease in the blood glucose level ($p < 0.05$) was observed in the diabetic rats treated with aqueous and ethanolic extracts of *V. amygdalina*.

	Day 1	Day4	Day 18
Group 1	67.2 ± 3.72^a	65.2 ± 7.67^a	65.0 ± 3.34^a
Group 2	72.2 ± 2.92^a	266.5 ± 31.39^b	178.3 ± 18.09^c
Group 3	72.2 ± 2.65^a	269.5 ± 21.47^b	187.3 ± 12.75^c
Group 4	68.2 ± 2.33^a	271.0 ± 40.92^b	180.0 ± 36.00^c

Table 2: Effects of Vernonia amygdalina extracts on the serum glucose level (mg/dl) of diabetic treated and untreated rats

Values are the mean ± SEM. Values with different superscript in the same column are significantly different ($p< 0.05$). Day 1- Before alloxan injection, Day 4- Four days after alloxan injection, Day 18- After treatment

Changes in the serum amylin and insulin levels of diabetic treated anduntreated rats are presented in table 3. The amylin level of the diabetic rats (group 2) was elevated compared to the normal control (group 1), although there was no statistical difference ($p > 0.05$). However, there was significant increase ($p < 0.05$) in the amylin levels of the diabetic rats treated with aqueous and ethanolic *V. amygdalina* extracts daily. The insulin level of group 2 (diabetic untreated rats) was significantly lower ($p < 0.05$)compared to group 1 (normal control). There were significant increases ($p < 0.05$) in the serum insulin levels of the diabetic treated rats (groups 3 and 4) as compared to the diabetic untreated rats (group 2).

Group	Amylin (pg/ml)	Insulin (µU/ml)
Group 1	1138.72 ± 233.71^a	0.17 ± 0.06^a
Group 2	1326.38 ± 231.44^a	0.04 ± 0.02^b
Group 3	1536.62 ± 185.85^b	0.67 ± 0.23^c
Group 4	1565.69 ± 149.54^b	0.41 ± 0.29^c

Table 3: Effect of aqueous and ethanolic extracts of Vernonia amygdalina on the serum amylin and insulin levels of alloxan- induced diabetic rats

4.Discussion

Diabetes mellitus results when there is dysfunctional insulin action. This condition is characterized by abnormally high glucose level (hyperglycemia). This might be due to a low level of insulin production, resistance to insulin at target tissue or T-cell mediated auto-immune attack (Rother, 2007). This study therefore aimed at determining the effect of both aqueous and ethanolic extracts of *Vernonia amygdalina* on the amylin and insulin levels released in alloxan induced diabetic rats.

Weight loss is one of the symptoms that characterized diabetes mellitus due to loss in muscles and adipose tissues resulting from excessive breakdown of tissue protein and over conversion of glycogen to glucose (Zink and Chaffin, 1998; Granner, 1996). In this study, the weight loss of the diabetic rats supports previous studies that there is significant weight reduction in untreated diabetic rats (Saidu *et al.,* 2010; Ahmed *et al*., 2005). The diabetic rats treated with aqueous and ethanolic extracts of *V. amygdalina* showed significant improvement in the body weight compared to the diabetic untreated rats which may be due to the protective role of the plant.

In this study, the diabetic rats treated with both aqueous and ethanolic extracts of *V. amygdalina* showed significant reduction in the serum glucose levels. This revealed both extracts possess hypoglycemic effect and could enhance blood glucose transportation to the peripheral tissue.According to Ong *et al*., (2011), V. amygdalina could decrease blood glucose by increasing GLUT 4 translocation and inhibit hepatic glucose-6- phosphatase.

The amylin level of the diabetic rats was elevated compared to the normal control, this could be due to the hyperglycemic effect of alloxan in which more amylin is released to prevent glucagon secretion in order to reduce the spike of hyperglycemia. Studies have shown that plasma amylin level is elevated in type 2 diabetic patients, which may result in hyperamylinemia and consequently aggregate into amyloid fibrils in islets of Langerhans, leading to loss ofβ- cells function (Abedini and Schmidt, 2013; Pillay and Govender, 2013; Hayden and Tyagi, 2002). The high concentration of amylin in the diabetic treated rats indicated both extracts could enhance the secretion of amylin to inhibit glucagon synthesis, inhibit lipolysis and improve glucose uptake to the peripheral tissues.

Diabetogenic drug such as alloxan selectively destroys the β-cells of the pancreas, thereby resulting in low/decreased secretion of insulin from the pancreas (Mir *et al*., 2013). In this study, the insulin level of the diabetic untreated rats was significantly lower ($p < 0.05$) compared to the normal controlindicating decreased secretion of insulin in the diabetic rats. The increased insulin levels of the diabetic treated rats show both aqueous and ethanolic extracts of V. amygdalina could improve

insulin sensitivity and subsequently reduce the hyperglycemic state of the diabetic rats. It may also be due to stimulation of β-cells and subsequent release of insulin and activation of the insulin receptor (Babu *et al*., 2003). As such, this study supports previous findings the V. amygdalina extracts possess antidiabetic properties (Ong *et al.,* 2011).

5.Conclusion

The results of this current study showed *Vernonia amygdalina* extracts possess hypoglycemic properties and enhances amylin and insulin secretion from the remaining β- cells of the pancreas after selective alloxan destruction, in order to reduce the blood glucose levels.

6.Acknowledgement

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