

Evaluation the Effect of *Vernonia amygdalina*. Del Leaves Ethanol Extract on Blood Sugar and its Synergism with Glibenclamide in Rats

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Abstract

Antidiabetic activity of *Vernonia amygdalina* and its possible synergism with glibenclamide was checked. Forty eight rats were used for the research, for hypoglycemic study of *V. amygdalina* alone, they were grouped into five of six rats each. Group 1 was the negative control and was administered distilled water orally. Groups 2, 3, and 4 were the treatment groups which received 100, 200 and 300 mg/kg body weight of the *V. amygdalina* extract respectively orally by intubation. Group 5 was the positive control group which received a known antidiabetic drug, glibenclamide. Diabetes was induced with alloxan. For the synergism study, another 18 rats grouped into 3 of six rats each was used. Both groups of glibenclamide only and glibenclamide plus *V. amygdalina* extract were dosed for 14 days orally by intubation, thereafter were sacrificed and blood collected from heart for analysis. There were 5 replicates grouped by weight throughout the study and both single and synergistic studies had the same controls. Effect of *V. amygdalina* extract was checked on blood glucose and its possible synergism with glibenclamide. All results in treatment groups were compared with the normal control at statistical confidence of $p < 0.05$. Result shows that *V. amygdalina* extract reduced blood glucose level in the test groups as dose of extract increased. Combination of *V. amygdalina* with glibenclamide demonstrated further deduction in blood glucose levels in the treatment rats groups. Therefore addition of *V. amygdalina* into glibenclamide increased efficacy in the diabetic rats. The interaction between *V. amygdalina* and glibenclamide in this work was additive and therefore synergistic.

Keywords: Alloxan, Blood glucose, Diabetes, Glibenclamide, Synergism, *Vernonia amygdalina*

تأثير مستخلص الاثانولي لاوراق نبات *V. amygdalina* على تركيز كلوكوز الدم وتأثيره التآزري مع glibenclamide في الجرذان

الخلاصة

تم دراسة فعالية مستخلص *ernonia amygdalina* كمضاد للسكري وإمكانية تعضيد فعاليتها باستخدامها مع دواء glibenclamide تم استخدام 48 جرذا مختبريا في هذا البحث حيث قسمت الى خمسة مجاميع كل مجموعة تحتوي على ستة جرذان المجموعة الأولى مجموعة السيطرة السالبة تمت تجريبيها فمويا بالماء المقطر فقط ، المجموعة الثانية جرعة مادة *Vernonia amygdalina* وبتركيز 100 ملغرام للكيلو غرام من وزن الجسم، المجموعة الثالثة جرعة مادة *Vernonia amygdalin* وبتركيز 200 ملغرام للكيلو غرام من وزن الجسم، المجموعة الرابعة جرعة مادة *Vernonia amygdalina* وبتركيز 300 ملغرام للكيلو غرام من وزن الجسم، اما المجموعة الخامسة فتم معاملتها كمجموعة سيطرة موجبة حيث تم تجريبيها بدواء glibenclamide الذي يعتبر كذلك علاج مسبب لفقر الدم ، تم استحداث الداء السكري بواسطة مادة الالوكسان ، ولعمل دراسة تآزرية تم استخدام 18 جرذا مختبريا تم تقسيمها إلى ثلاثة مجاميع كل مجموعة تتكون من ستة جرذان المجموعة الأولى جرعت بمادة glibenclamide اما المجموعة الثانية فقد جرعت مادة *Vernonia amygdalina* مع glibenclamide لمدة 14 يوم بعد ذلك تم التضحية بجميع حيوانات التجربة وتم جمع الدم من القلب مباشرة لإجراء اختبار قياس نسبة سكر الدم. أظهرت النتائج بعد مقارنة المجموعات المعاملة مع مجموعة السيطرة إحصائيا ($p < 0.05$) أن المجموعات المعاملة ب مستخلص ال (*Vernonia amygdalina*) أظهر انخفاضا معنويا بالمقارنة مع مجموعة السيطرة السالبة وكذلك المجموعة المجرعة بالمزيج التآزري (*Vernonia amygdalina*) مع (*glibenclamide*) أظهر كذلك انخفاضا معنوي ($p < 0.05$) اعلى في مستويات كلوكوز الدم.

Introduction

Vernonia amygdalina (Compositae) is a small shrub that grows in Africa and other parts of the world. In some parts of West Africa like Nigeria, the plant has been domesticated. The English name is bitter leaf while the Igbo tribe in Nigeria call it *Olugbu*, the Tivs, *ityuna*, Edos, *Oriwo* and the Hausas of Northern Nigeria call it *Chusar-doki*. Leaves of *V. amygdalina* are used for preparing the popular bitter-leaf soup while the juice or extract serves as a tonic drink. *V. amygdalina* is used in Nigeria both treatment of diseases and nutritional purposes (1). It has bitter taste associated with anti-nutritional factors like alkaloids, saponins, tannins and glycosides (2) and enriched with micro and macroelements (3).

V. amygdalina is used in traditionally as antihelminthic, anti-malaria and as laxative herb (4). Leaf of *V. amygdalina* has been shown to be useful as a remedy for gastrointestinal discomfort and stomach upset (5). It has blood sugar lowering effect in experimental rabbits (6), serum cholesterol lowering effects (7). *V. amygdalina* has been shown to increase urinary and faecal output in mice fed a diet incorporated with the extract, (3). *V. amygdalina* has protective effects against the toxic effects of aflatoxin B₁ exposure (8) and may help in kidney clearance functions (9).

The composition of the vegetable *Vernonia amygdalina* has been shown to affect uterine motility (10). For centuries people have used plants for healing. Plants are important sources of novel pharmacologically active compound, (11) (12) Traditional medicine has assisted in fighting many difficult health challenges affecting Africans but lack scientific proof of its efficacy. Consumption of high quantities of *V. amygdalina* is observed to have no hepatotoxic effect (13).

(14) investigated aqueous extracts of *V.*

amygdalina in streptozotocin-induced hyperglycemic rats and reported reduction in fasting blood glucoses levels in the diabetic rats.

The leaf extracts have both hypoglycemic and hypolipidaemic properties and could be used in managing diabetics mellitus (6). In the report of (7) the antidiabetic effects of aqueous extract of leaves of *V. amygdalina*. World health organization (15) define diabetes as fasting blood glucose level greater than 140 mg/dl or greater than 200 mg/dl, (14) in experimental rats. It is a metabolic disease characterized by hyperglycemia and glycosuria due to lack of insulin. (16)

V. amygdalina helps in keeping healthy blood glucose levels (14), sugar lowering effect in experimental rabbits (6), serum albumin and cholesterol lowering effects (7), blood lipid lowering effect in rats fed high cholesterol diet (17).

Since *V. amygdalina* has antidiabetic effect and used commonly as vegetable in soup and tonic drinks, it could be beneficial when used together with conventional antidiabetic agent to increase efficacy and reduce toxicity associated with conventional drugs. This is why we undertake this research.

Materials and methods

Plant Materials

Leaves of *V. amygdalina* were collected from the University environment in Umudike, Nigeria and was identified by Prof. M. C. Dike at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria. The study was carried out during early rainy season (March-April).

Preparation of Plant Extract

The identified leaves of *V. amygdalina* was dried under shade for 10 days and grinded to a coarse powder using manual grinder (Corona-Landers C 1A SA). Extraction was done by Soxhlet method described by (18) and 35g of coarse powdered sample was

introduced into the extraction chamber using 80% ethanol as solvent. Throughout the extraction time of 48 hrs the temperature was kept at 70⁰ C. The extract was concentrated in an oven at 300 C and the dried extract weighed and kept in a labelled sterile specimen bottle for the work. Different doses of 100, 200 and 300 mg/kg body weight was prepared and administered to rats in group 2, 3, and 4 respectively. These doses were calculated from a stock solution dissolved in distilled.

Chemicals

Alloxan was used in this study and was obtained from Sigma and Alderich USA. Other reagents/chemicals used were obtained within Nigeria and were of analytical grade.

Experimental Animals

Adult albino rats weighing (140 to 250 g) were purchased from University Farm. Approval was obtained from College of Vet Medicine, Michael Okpara University of Agriculture Umudike, Nigeria, in line with the guidelines for the care and use of laboratory animals as given by the National Research Council (19). The rats were acclimatized and fed ad *libitum*.

Experimental Design

Thirty rats were used for the research; they were grouped into five of 6 rats each. Group 1 was the negative control group and was administered distilled water orally by intubation. Groups 2, 3, and 4 were the treatment groups which received 100, 200 and 300 mg/kg orally by intubation. body weight of the *V. amygdalina* extract respectively. Group 5 was the positive control group which received a known antidiabetic drug, Glibenclamide orally. Both groups of glibenclamide only and glibenclamide plus *V. amygdalina* extract were dosed for 14 days orally by intubation, thereafter were sacrificed and blood collected from heart for analysis. There were 5 replicates grouped by weight throughout the study and both single and synergistic had the same control. The rats were dosed for 14 days, thereafter were

sacrificed by cardiac puncture and blood collected for analysis. The effect of *V. amygdalina* extract was checked on blood glucose and its synergism with glibenclamide.

Experimental Diabetes Induction

The method of (20) was adopted. The animals were fasted for 16–18 hours with free access to water before the induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of Alloxan Monohydrate (Sigma St Louis, M.O., USA) dissolved in 0.9% V/V normal saline solution at a dose of 150 mg/kg body weight (21). The diabetes was assessed in alloxan induced rats by determining the blood glucose concentration using one touch glucometer and Accu-check strips at day 1 and day 3 after injection of alloxan. The Wistar rats that recorded an elevated blood glucose concentration above 240 g/dl were considered diabetic and were selected for the study.

Blood Glucose Levels determination

The modified procedure of Folin Wu (22), based on the glucose oxidase principle was adopted. To determine blood sugar level of the rats. Blood samples were collected from the tail artery and a drop allowed to touch the sensor part of one touch glucometer strips. The values obtained were recorded in mg/dl. The blood glucose concentration was sampled at intervals of before induction, day 1, day 3 and day 7 of treatment, respectively. This method was used to confirm diabetic induction. After 14 days, rats were sacrificed by cardiac puncture and blood collected for analysis.

Statistical Analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 20. Values were expressed as mean \pm Standard Error of Mean (SEM) and were further subjected to one - way analysis of variance (ANOVA) for

comparing doses with normal control. Duncan post-hoc test was used to separate the mean that showed significant difference. The statistical confidence was set at $p < 0.05$.

Results and discussion

Result Presentation One

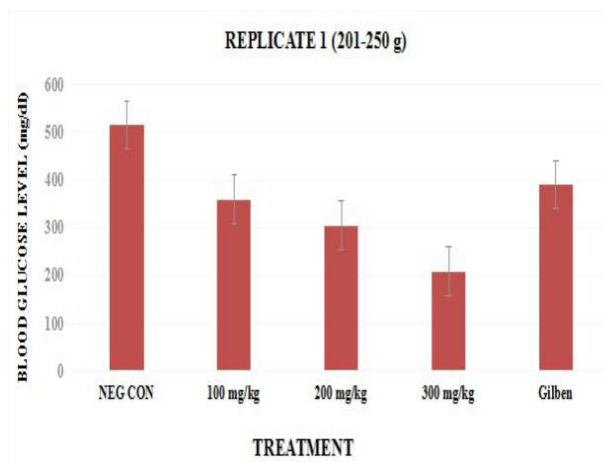


Fig 1 Shows effect of *V. amygdalina* on Alloxan induced diabetes.

Replicate 1 weighing 201-250 g shown in Fig 1, represent the graph of antidiabetic effect of *V. amygdalina* on Alloxan induced diabetes. As dose increased there was reduction in glucose level from 362.00 ± 1.15 mg/dl, 280.33 ± 0.88 mg/dl and 250.00 ± 0.66 mg/dl when compared to the negative control 407.66 ± 0.88 mg/dl. The positive control group was 391.13 ± 0.57 mg/dl.

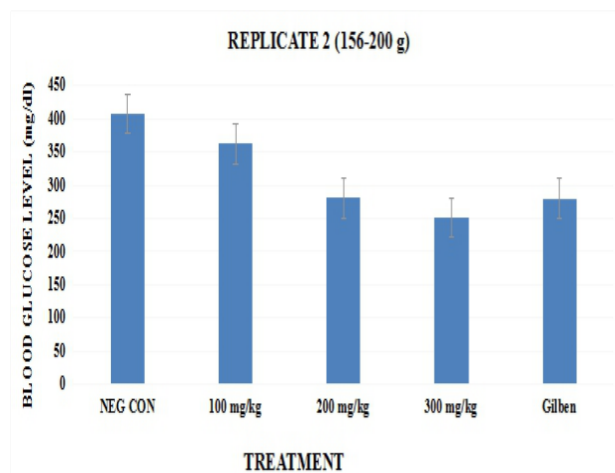


Fig 2 Shows effect of *V. amygdalina* on Alloxan induced diabetes.

Replicate 2 weighing 156-200 g shown in Fig 2, represent the graph of antidiabetic effect of *V. amygdalina* on alloxan induced diabetes in Wistar rats. As dose increased there was a reduction in glucose level from 362.00 ± 1.15 mg/dl, 280.33 ± 0.88 mg/dl and 250.66 ± 0.66 mg/dl when compared to the negative control 407.66 ± 0.88 mg/dl. The positive control group was 316.13 ± 0.57 mg/dl.

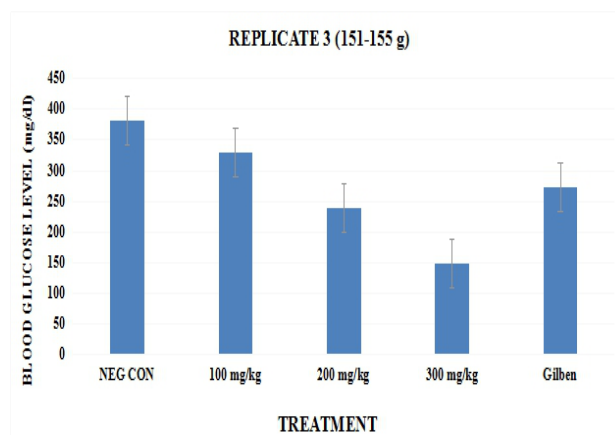


Fig 3 Shows effect of *V. amygdalina* on Alloxan induced diabetes.

Replicate 3 weighing 151- 155 g shown in Fig 3, represent the graph of antidiabetic

effect of *V. amygdalina* on alloxan induced diabetes in Wistar rats. As dose increased there was reduction in glucose level from 330.00 ± 0.57 mg/dl, 240.33 ± 0.57 mg/dl and 149.33 ± 0.33 mg/dl when compared to the negative control 407.66 ± 0.88 mg/dl. The positive control group was 274.00 ± 0.57 mg/dl.

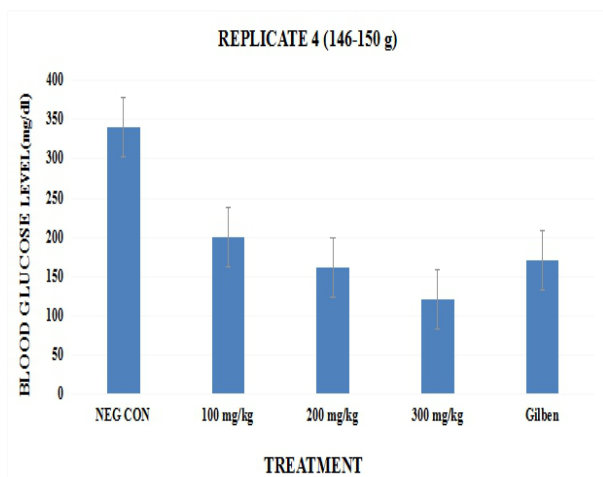


Fig 4 Shows effect of *V. amygdalina* on Alloxan induced diabetes.

Replicate 4 weighing 146- 150 g shown in Fig 4, represent the graph of antidiabetic effect of *V. amygdalina* on alloxan induced diabetes in Wistar rats. As dose increased there was a significant reduction ($p < 0.05$) in glucose level from 201.00 ± 0.57 mg/dl, 161.66 ± 0.88 mg/dl and 120.66 ± 0.33 mg/dl when compared to the negative control 407.66 ± 0.88 mg/dl. The positive control group was 170.33 ± 0.33 mg/dl.

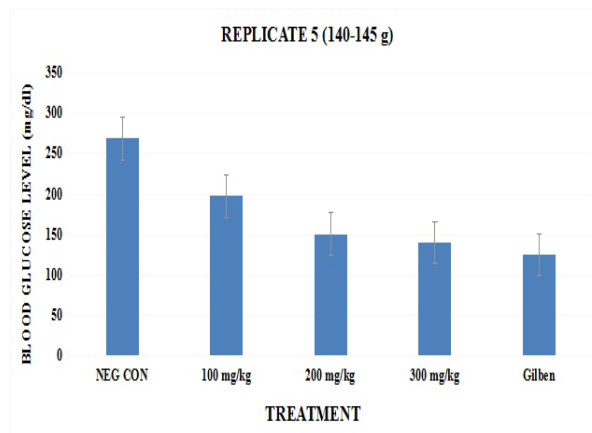


Fig 5 Shows effect of *V. amygdalina* on Alloxan induced diabetes.

In replicate 5 weighing 140- 145 g shown in Fig 5, represent the graph of antidiabetic effect of *V. amygdalina* on alloxan induced diabetes in Wistar rats. As dose increased there was significant reduction in glucose level from 198.00 ± 0.57 mg/dl, 151.33 ± 0.33 mg/dl and 140.66 ± 0.66 mg/dl when compared to the negative control 407.66 ± 0.88 mg/dl. The positive control group was 126.00 ± 0.57 mg/dl.

Result Presentation Two

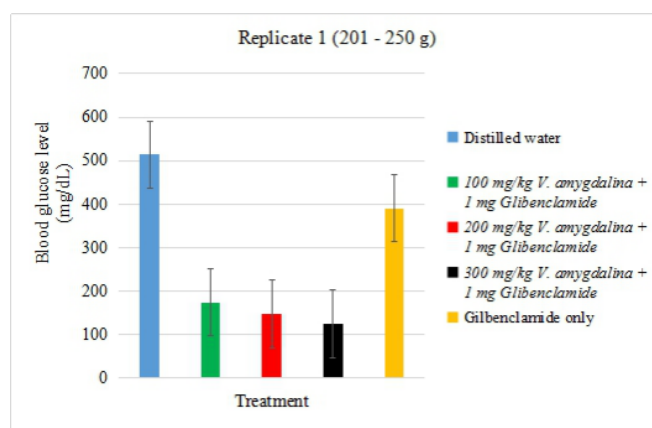


Figure 6: Synergistic effect of *V. amygdalina* and Glibenclamide on Alloxan induced diabetes.

Further reduction in glucose level when *V.*

amygdalina was administered in combination with glibenclamide. In replicate 1 weighing 201-250 g, the reduction in glucose was 159.66 ± 0.66 mg/dl, 105.33 ± 0.88 mg/dl and 109.66 ± 0.57 mg/dl when compared to the negative control 514.66 ± 1.25 mg/dl. The positive control group was 391.00 ± 0.57 mg/dl.

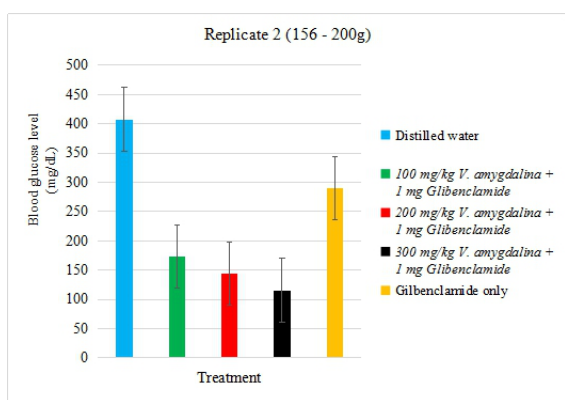


Figure 7: : Synergistic effect of *V. amygdalina* and Gilbenclamide on Alloxan induced diabetes.

Further reduction in glucose level when *V. amygdalina* extract was administered in combination with glibenclamide. In replicate 2 weighing 156-200 g, the reduction in glucose was 173.66 ± 2.33 mg/dl, 144.66 ± 2.40 mg/dl and 115.66 ± 2.84 mg/dl when compared to the negative control 407.66 ± 0.88 mg/dl. The positive control group was 280.00 ± 0.57 mg/dl.

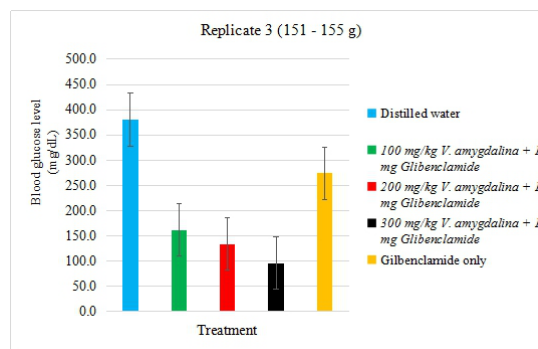


Figure 8. Synergistic effect of *V. amygdalina* and Glibenclamide on Alloxan induced diabetes.

Further reduction in glucose level when *V. amygdalina* extract was administered in combination with glibenclamide. In replicate 3 weighing 151-155 g, the reduction in glucose was 162.00 ± 1.15 mg/dl, 133.66 ± 1.20 mg/dl and 96.00 ± 1.15 mg/dl when compared to the negative control 380.33 ± 0.88 mg/dl. The positive control group was 274.00 ± 0.57 mg/dl.

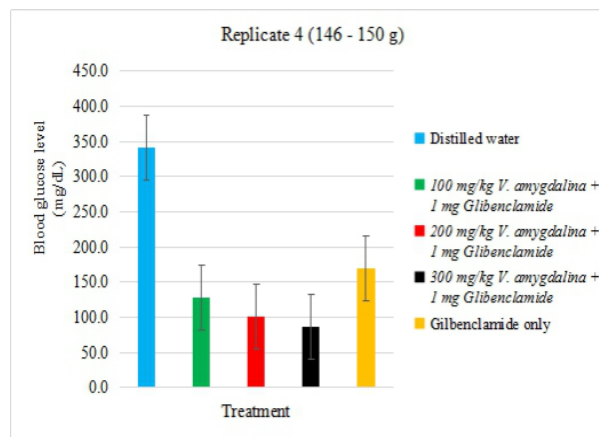


Figure 9. Synergistic effect of *V. amygdalina* and Glibenclamide on Alloxan induced diabetes.

There was more reduction in glucose level when *V. amygdalina* extract was administered in combination with glibenclamide. In replicate 4 weighing 146-

150 g, the reduction in glucose was 128.33 ± 0.88 mg/dl, 100.66 ± 1.76 mg/dl and 88.66 ± 0.66 mg/dl when compared to the negative control 341.00 ± 0.57 mg/dl. The positive control group was 170.33 ± 0.33 mg/dl.

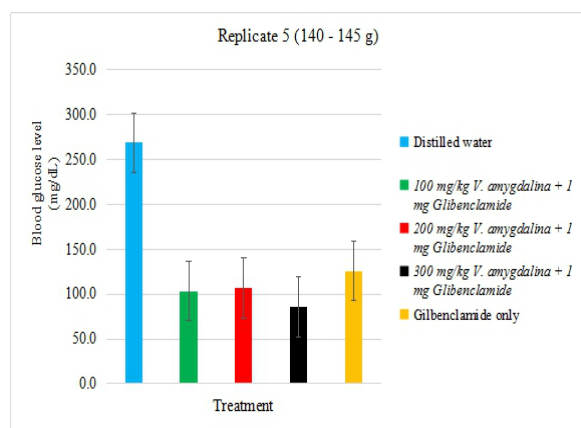


Figure 10. Synergistic effect of *V. amygdalina* and Glibenclamide on Alloxan induced diabetes.

There was more reduction in glucose level when *V. amygdalina* extract was administered in combination with glibenclamide. In replicate 5 weighing 140-145 g, the reduction in glucose was 103.33 ± 0.66 mg/dl, 107.00 ± 0.57 mg/dl and 85.66 ± 2.96 mg/dl when compared to the negative control 269.00 ± 0.57 mg/dl. The positive control group was 126.00 ± 0.57 mg/dl.

Screening for new antidiabetic drug of plant origin to assist the pharmaceutical industries is important. Plants remains the best alternative because they contain substances which are safer for use on diabetes mellitus (23). Type 2-diabetes was induced in rats by a single intra-peritoneal injection of alloxan monohydrate at 150 mg/kg body weight. Alloxan is toxic to islet cells of pancreas so can cause diabetes in

many animal species by damaging insulin β -cell of islet of Langerhans resulting in decreased insulin release, consequently decrease utilization of glucose by the tissues thus elevating blood glucose level (20).

This research was designed to authenticate the claim by natives that *V. amygdalina* extract can be used to reduce the blood glucose level of diabetic patients. So administration of *V. amygdalina* extract at the graded dosage used on alloxan-induced diabetic rats caused reduction of elevated glucose level (anti-hyperglycemic effect).

The recovery of diabetic rats from extract of *V. amygdalina* may be from remaining beta cells of Langerhans due to insulin release from the existing cells of the pancreas, which may have stimulated insulin secretion and regeneration of beta cells or activation of enzymes responsible for glucose utilization (24).

This research shows that *V. amygdalina* and glibenclamide can work synergistically. Glibenclamide has side effects and complications in some patients like weight gain, abdominal pain, constipation, nausea, vomiting, diarrhoea, and loss of appetite. Other side effects such as headache, dizziness, drowsiness can occur. Therefore *V. amygdalina* though less effective as compared with glibenclamide should be recommended instead of glibenclamide in animal production because glibenclamide has a withdrawal period. *V. amygdalina* may be safe when traces appear in meat and milk production. Leaves of *V. amygdalina* are readily available and cheaper than glibenclamide. In drug administration, *V. amygdalina* does not need expertise because most farmers in the rural areas are illiterates and it has no side effects (10)

Conclusion

V. amygdalina demonstrated antidiabetic potential. Therefore, corporation of *V. amygdalina* into glibenclamide will increase efficacy in diabetic patients. The interaction between *V. amygdalina* and glibenclamide in this work was additive and therefore synergistic.

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