

Full Length Research Paper

Antidiabetic activity and acute toxicity evaluation of aqueous leaf extract of *Vernonia amygdalina*

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The present study was aimed to explore the therapeutic dose for antidiabetic activity and toxicological evaluation of *Vernonia amygdalina* (*Va*) aqueous extract in alloxan-induced diabetic rats. Aqueous extract of leaves of *Va* was administered to alloxan induced diabetic rats in a dose of 150 and 300 mg/kg (orally) daily for 14 days. After this period, blood glucose level, haematological parameters and liver enzymes activities were evaluated. Also evaluated were food and water intake, urine output and body weight of the animals. The toxic effect of the aqueous leaves extract was evaluated by determining the LD₅₀. Oral administration of the extract at graded doses of 150 and 300 mg/kg body weight showed significant decrease in the blood glucose level in diabetic rats ($P < 0.05$). The defects in haematological and enzyme activities in the diabetic animals were restored. We concluded that at doses of 150 and 300 mg/kg, *Va* extract exhibited anti-hyperglycemic effect and showed statistically significant differences ($p < 0.05$) in all the parameters evaluated. There was a significant improvement ($P < 0.05$) in the weight of the diabetic rats, food intake and a decrease in the urine output. This study illustrates the potential usefulness of this extract and its safety on a vital organ of the body.

Key words: Alloxan, *Vernonia amygdalina*, diabetics, aqueous extract, rats, safety.

INTRODUCTION

Diabetes mellitus is the clinical condition of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Obimba et al., 2014; Ozougwu et al., 2013). The chronic hyperglycemia caused by diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (International Diabetes Federation (IDF),

2009). There are two types of diabetes: Type 1 diabetes, previously called insulin-dependent diabetes mellitus or juvenile-onset diabetes which accounts for up to 10% of all diagnosed diabetes which is caused by lack of insulin secretion by β -cells of the pancreas. Type 2 diabetes was previously called non-insulin-dependent diabetes mellitus or adult-onset diabetes which may account for between 90 to 95% of all diagnosed cases of diabetes (Obimba et

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al., 2014; Ozougwu et al., 2013). Type 2 diabetes mellitus has become a significant health problem in both developed and developing countries. IDF has estimated that 285 million people around the world have diabetes. This total is expected to rise to 438 million within 20 years (World Health Organization, (WHO), 2003). The non-pharmacological means (diet and exercise) and/or the pharmacological means (insulin and oral hypoglycaemics) have been used in the management of diabetes mellitus. The obvious limitations of oral administration of antidiabetic agents, especially with insulin, have necessitated a search for alternatives among the arsenal of herbs available to man (Edwin et al., 2006). A large number of herbs, spices and other plant materials have been described for the management of diabetes throughout the world (Ojiako and Nwanjo, 2006). It was in this light that the World Health Assembly adopted among its resolutions the support of national traditional medicine program, drawing attention to herbal medicines as being of great importance to the health of individuals and communities (Emeje et al., 2011).

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived from plants. *Vernonia amygdalina* (family, Compositae) is a valuable shrub that is wide spread in East and West African countries (Izevbigie, 2003). In Nigeria, it is commonly known as "bitter leaf" because the leaves and stem have a bitter taste when chewed. Its leaves were used as a popular vegetable for soups particularly among the ethnic groups in Nigeria. The organic fraction extracts of the plant was shown to possess cytotoxic effects on human carcinoma cells of the nasopharynx (Okolie et al., 2008). It is effective against gastrointestinal disorders (Akah and Ekekwe, 1995) including amoebic dysentery (Moundipa et al., 2005) possibly due to its antimicrobial and antiparasitic activities (Gray et al., 2000; Muraina et al., 2010). Its anti-thrombotic and anticoagulant properties have also been evaluated (Akah and Ekekwe, 1995). Oral administration of the aqueous leaf extract of the plant was found to relieve pain and to lower body temperature (Gray et al., 2000). *V. amygdalin* is also used traditionally as an antidiabetic remedy in Nigeria by the traditional herbal practitioners (Muraina et al., 2010; Park, 2007; Atawodi, 2005). Several workers have provided some scientific proofs in support of this practice (Abdulazeez et al., 2013; Iwuji et al., 2010) but, available scientific reports on the actual dose needed for management of diabetic conditions and the possible mechanism of action of this plant were not detailed enough. Thus, we conducted this study to further investigate the effective dose for anti-hyperglycemic effect and to a less extent its safety on a vital organ of the body.

MATERIALS AND METHODS

Preparation of crude extract

Pesticide-free fresh leaves of *V. amygdalina* were collected from

Nsukka, Enugu State, Nigeria and authenticated at Bioresources Development and Conservation Program (BDGP), Nsukka, Nigeria where voucher samples were kept for reference. Healthy fresh leaves were sorted, washed to remove debris and dust particles without squeezing and then air-dried for seven days (Momoh et al., 2013). The dried leaves were milled into a coarse powder from which 25 g was soaked with 500 ml of distilled water in a beaker and the mixture shaken on the laboratory bench for 24 h before filtering (Whatman No. 1 filter paper). The filtrate was evaporated using a rotary evaporator to obtain a solid residue (5.0 g) called the aqueous extract which correspond to a percentage yield of 20%. This procedure was carried out in quadruplicate to obtain a total of 20.0 g of aqueous extract. Appropriate weights of the residue were reconstituted separately in distilled water to give the required doses of 100, 150 and 300 mg/kg body weight used in the present study.

Phytochemical tests on the extract

Some phytochemical tests were carried out on the *V. amygdalina* extract to determine the presence of saponins, flavonoids, alkaloids, proteins, carbohydrates, glycosides and tannins using standard methods (Sofowora, 2006).

Preliminary evaluation of hypoglycemic activity of extract in normal healthy albino rats

Twenty normal Albino rats, fasted overnight, were divided into four groups of five rats each, and used in the experiment. Group A served as control, and received the vehicle (distilled water only) while animals of groups B, C and D received variable doses of 100, 150 and 300 mg/kg, respectively, of extract suspended in distilled water. The blood samples were collected from the lateral vein of the tail of the animals at 2, 4, 6, 8 and 12 h after administering the extract and the sugar levels were measured. These were compared to the initial blood glucose level.

Experimental design

Albino rats (180 to 220 g) of either sex were used for the study. The animals were procured from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, and were allowed to acclimatize to the new environment for a period of two weeks prior to the study. Rats housed in cages were kept in a room with controlled temperature (20 to 22°C) and a 12 h day-night cycle. Fresh solution of alloxan monohydrate (Sigma, USA) was prepared just prior to injection. A stock solution of it was made by dissolving all in normal saline (0.9% w/v NaCl) at a concentration of 100 mg/mL (Dhasarathan and Theriappan, 2011). After being starved overnight, a volume equivalent to 1 mL of the stock solution was administered intra-peritoneally to the animals after which the blood glucose levels were measured for days using a glucometer (ACCU-CHEK, Roche, USA). Food consumption, water intake and urine volume were similarly evaluated daily. The rats were considered diabetic when the blood glucose level was raised above 200 mg/100 mL of blood (Cetto et al., 2000) after 3 days post-alloxan administration. All of the animal experiments adhered to the Ethical Guidelines of Animal Care and Use Committee (Research Ethics Committee) of Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria.

Oral administration of graded dose of extract to diabetic rats

Twenty (20) Albino rats were used for the study. They were divided into four groups of five rats per group. Based on an initial normoglycemic study, 150 and 300 mg doses were selected for the

Table 1. Phytochemical analysis of *Vernonia amygdalina* extract.

Test	Present/absence	Inference
Alkaloids	+	Present
Cyanogenic glycosides	-	Absent
Cardiac glycosides	++	Present
Anthracene glycosides	-	Absent
Steroidal glycosides	++	Present
Saponins	+	Present
Tannins	++	Present
Flavonoids	+	Present
Proteins	+	Present
Carbohydrates	+	Present

+ indicates presence of phytochemical secondary metabolite; - indicates absence of phytochemical secondary metabolite.

antidiabetic evaluation. The first group marked A, received distilled water (negative control, 1.5 mL orally). The groups marked B and C received the extract at 150 and 300 mg dispersed in distilled water (1.5 mL orally), respectively, while that marked D received glibenclamide dispersed in distilled water (2.0 mg/Kg orally). The extract was administered once daily by gastric intubation for 14 consecutive days.

Acute toxicity evaluation

The acute toxicity studies were carried out based on Lorke's method (1983) (with slight modifications) on adult male Wistar rats of an average weight of 220 g. The animals were housed in air-conditioned quarters under a photoperiod schedule of 12 h light/12 h dark. They were fed on standard animal pellets and had free access to water *ad libitum*. The rats were randomized into two groups (i and ii) of five rats each and were orally administered graded doses of the extract 3,000 and 6,000 mg/kg body weight, respectively. The doses were 20 times the most effective dose of the aqueous extract of *V. amygdalina* used in the antidiabetic evaluation. They were observed for signs of toxicity that included paw-licking, stretching, response to stimuli and mortality for the first 4 h and thereafter daily for seven days. Food consumption, water intake and urine output were also examined for 24 h. The lethal dose was calculated as the geometric mean of doses that caused 0 and 100% mortality, respectively.

Effect on liver enzymes and haematological parameters

Haematological parameters

Haematological analysis was performed using an automatic haematological analyzer (Abacus Junior, Germany). Haemoglobin (Hb) count, total white blood corpuscles (WBC) and packed cell volume (PCV) were specifically determined.

Liver enzymes activity

A 3 ml volume of blood collected in a plain bottle or serum extractor was used for the study. The blood was allowed to stand in an undisturbed bench for 1 h away from sunlight; this was followed by spinning for 5 min. Serum was separated from the clotted red cells, and the resulting supernatant used for the assessment of liver

integrity. Using a 3.2 mL automated pipette serum was dropped on the sample spot of each LFT parameter strips (ALP) Aspartate and Alanine aminotransferases strip) and analysed using a Reflotron-Plus machine (Model: SN747461).

Statistical analysis

Statistical analysis was performed using SPSS statistical package. Mean and standard errors for all data were calculated. For batch comparisons, the Student's t-test was used to determine statistically significant differences at $P \leq 0.05$.

RESULTS

The results from the study showed that the aqueous leaf extract of *Va* extract was positive for alkaloids, saponins, flavonoids, phenolics, tannins, cardiac glycosides and steroids (Table 1).

Effect of extract on fasting blood glucose level of normal healthy rats

Results in Table 2 reveal the hypoglycemic effect of graded doses of aqueous extract of *V. amygdalina* leaves on blood glucose level (BGL) of normal rats. Rats treated with 300 mg/kg of extract showed a maximum fall of 2.0% in BGL at 8 h of oral administration, whereas reduction of less than 2.0% were observed with the doses of 100 and 150 mg/kg, respectively, at 12 h of administration. Statistically, the extract produced no significant decrease on the BGL of normoglycemic (non-diabetic) rats ($P > 0.05$).

Effect on the pre-and post polytriads symptoms and on body weight

As shown in Table 3, there were significant differences in

Table 2. Effect of graded doses of aqueous extract of *Vernonia amygdalina* leaves on normoglycemic rats (mean \pm S.D, n=3).

Batch/treatment	Dose mg	Blood glucose level (%) at times (h)					
		0	2	4	6	8	12
A (negative control)	DW	100	99.8 \pm 0.2	101 \pm 0.2	103 \pm 0.2	100 \pm 0.0	102 \pm 0.3
B (extract)	100	100	99.1 \pm 0.1	98.0 \pm 0.1	97.0 \pm 0.3	99.0 \pm 0.2	98.9 \pm 0.0
C (extract)	150	100	95.4 \pm 0.0	98.7 \pm 0.4	98.0 \pm 0.4	98.0 \pm 0.1	100 \pm 0.1
D (extract)	300	100	99.1 \pm 0.2	98.3 \pm 0.2	99.4 \pm 0.3	98.0 \pm 0.2	98 \pm 0.3

Table 3. Effect on polytriad symptoms and on body weight before and after treatment with the extract in normo and hyperglycemic rats.

Parameter	Before treatment		After treatment with extract	
	Normoglycemic	Diabetic	Normoglycemic	Diabetic
Urine output (ml)	6.20 \pm 1.2	97.1 \pm 1.4a*	8.10 \pm 1.2	8.6 \pm 0.2a*
Weight (g)	180 \pm 0.4**	114 \pm 0.2a*	182 \pm 0.4**	176 \pm 0.2a*
Food intake (g)	23.0 \pm 0.6	83.0 \pm 2.3a*	24.0 \pm 0.1	27.0 \pm 2.3a*
Water intake (ml)	21.0 \pm 1.3*	123 \pm 1.2a*	23.4 \pm 0.1*	25 \pm 1.2a*

a* Indicates significant difference at $p < 0.05$ among the diabetic treated group, and ** values were not significant ($P > 0.05$) when compared to non-diabetic before and after treatment. All the treated diabetic rats showed significant improvement ($P > 0.05$) compared to the normoglycemic rats before and after. Values are mean \pm SD (n = 3).

all the parameters evaluated (water and food intake, urine volume output, and weight gain) between the groups of diabetic and non-diabetic animals. Studies have shown that food intake, urine output and decrease in body weight are associated to diabetes conditions due to impaired metabolic pathway that greatly affected the physiological wellbeing of the body. In this research, aqueous extract of *Va* administered to the diabetes groups successfully restored the anomalies comparably to the control normoglycemic rats. The food consumption of the diabetic rats increased approximately 15%, although these animals showed less body weight (weight = 114.0 g) compared to the group of non-diabetic animals (body weight = 180.2 g). Treatment of the diabetic animals with *Va* extract led to a significant change ($P > 0.05$) in their consumption of food and water as well as the urine output and gain in body weight, comparable to the untreated non-diabetic rats (before administration of the extract). After the administration of the extract, there was a significant increase ($P < 0.05$) in the body weight of the diabetic group comparable to the body weight of the control group. However, there were no significant change ($P > 0.05$) in the urine output, food intake and gain in the body weight of the normoglycemic group after treatment with the aqueous extract (Table 3). The results clearly indicate that the diabetic conditions which cause an increase of food and water consumption and urine output, which ultimately lead to weight loss were generally reversed after the treatment. On physical examination, the changes in non-diabetic and diabetic rats were apparently distinctive because, apart from the

thinness of diabetic rats, the rats also maintained a quiet look and showed a slow response to external stimuli, that is, touch. All these observations were restored in all the treated groups regardless of the concentration of the extract administered. However, the recovery rate was dose-dependent.

In vivo experiment

As shown in Figure 1, *V. amygdalina* extract orally administered in diabetic rats allowed a significant decrease of glycemia compared to rats treated with water alone. The rats that received DW continued to have elevated blood glucose levels within the first 4 days, which may be due to force feeding as this can increase the glucose level. The decrease in the blood glucose level that was observed was due to the fasting conditions which caused a decrease in the blood glucose level. The extract (150 and 300 mg) dose-dependently lowered the blood glucose levels of the rats. Maximum blood glucose lowering (100 to 51%) was encountered in group C that was treated with 300 mg of the extract on the 6th and 12th day, and was comparable to the blood glucose reduction (100 to 54%) encountered in the group received a standard drug (glibenclamide) at the 4th days and later continued to show a slight increase throughout the period of this investigation (Figure 1). Although, it was observed that the standard drug showed relatively more anti-glycemic effect within 4 days, the extract was able to maintain a persistent steady decrease in BGL in the

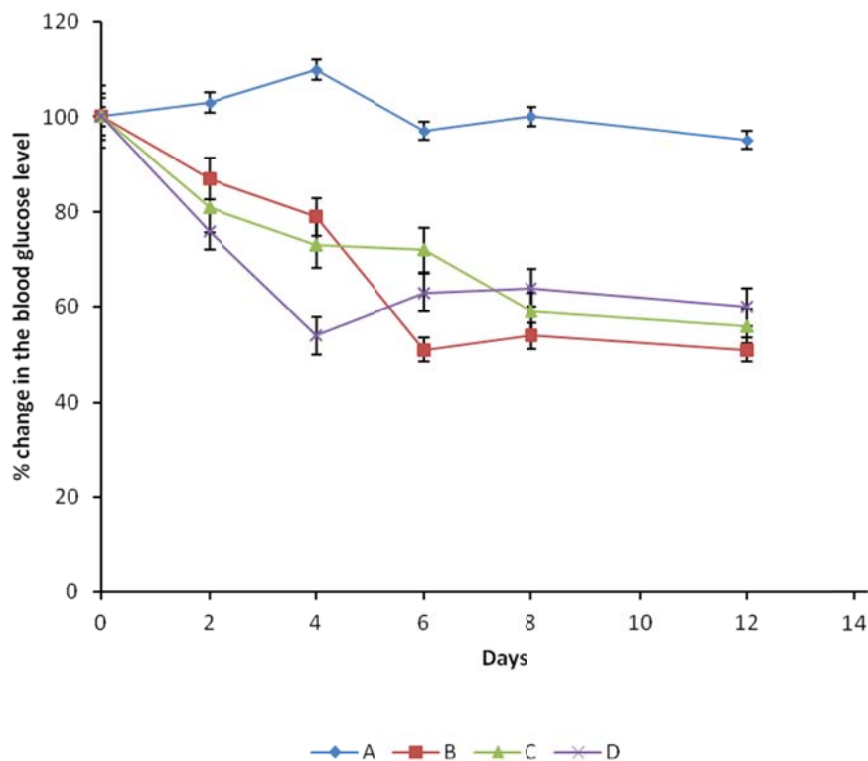


Figure 1. Blood glucose levels of alloxan-induced diabetic rats after oral administration of graded doses of the extract: (A) = distilled water (negative control, (B) = 150 mg extract, (C) = 300 mg extract and (D) = Glibenclamide 2.0 mg/kg (positive control).

Table 4. Effect of extract on haematology and liver enzymes activities (values = \pm SD).

Group	Dose (mg)	Haematological parameter			Liver enzyme activity (U/L)		
		PCV %	WBC ($\times 10^9$)	Hb g/dl	AST	ALT	ALP
A (DC)	DW	35.3 \pm 0.21	8.4.3 \pm 0.2	13.6 \pm 0.11	132.9 \pm 0.21	201.4 \pm 0.11	239 \pm 0.31
B	100	57.0 \pm 0.14	8.9.0 \pm 0.3	16.1 \pm 0.13	101.0 \pm 0.11	104.0 \pm 0.21	219 \pm 0.14
C	150	64.2 \pm 0.11	8.5.0 \pm 0.1	17 \pm 0.12	97.1 \pm 0.31	94.0 \pm 0.13	179 \pm 0.21
D	300	65.0 \pm 0.12	8.5.0 \pm 0.2	17.2 \pm 0.11	111.0 \pm 0.14	64.0 \pm 0.11	181 \pm 0.15
E	DW	69.01 \pm 0.22	7.40 \pm 0.5	20.1 \pm 0.21	62.9 \pm 0.21	41.1 \pm 0.11	142.1 \pm 0.21

DC=Diabetes control; DW= Distilled water; NC= non-diabetes control; B, C and D tests batches at different concentrations. (n = 3); \pm SD = standard deviation.

glycemic state, which is one of the desired effects in the clinical management of diabetic conditions.

Effect of the extract on haematology and liver enzymes

Previous studies have shown that the investigation of the acute toxicity is the first step in the toxicological study of an unknown substance. Although, the plant in question has been in use as a source of food in Nigeria and many other African countries, it is very important that the safety

of this plant should be evaluated before proceeding to the formulation proper and also to provide the scientific justification for the medicinal utilization of this plant. The result of the haematological evaluation on the extract based on the evaluated parameters (PCV, WBC and Hb) showed no significant changes ($P > 0.05$) in the hematological and liver enzymes activities as shown in Table 4. Diabetic conditions resulted in the decrease of the haematological parameters such PCV, Hb and WBC due to glycosylation that occurred as a result of excess sugar in the blood as seen in the diabetic control (DC) group.

DISCUSSION

Throughout the world, diabetes is the fastest growing metabolic disorder and is considered as a heterogeneous group of diseases characterized by major causes affecting cardiovascular, renal, neurological and ophthalmic systems (Chakkarwar and Manjrekar, 2005). Currently available synthetic oral antihyperglycaemic agents may be associated with an increased risk of unwanted effects on prolonged use (Edwin et al., 2006). So, there is need to investigate newer herbal medicines which have less side effects, easy availability and are economical (Shah et al., 2006). The age long use of herbal medicines in the management of diabetic conditions is a common practice in many countries across the globe including Nigeria. Therefore, the need to substantiate the folkloric claim of *V. amygdalina* as an antidiabetic agent using rat models is imperative. The results show that there was statistically significant P reduction ($P < 0.05$) not only in the glucose level but also in the associated polytriads symptoms. Other related complications including weight loss were also improved significantly ($P < 0.05$) in the treatment groups over time as compared to the negative control in the present study. Alloxan became the first diabetogenic chemical agent when scientists accidentally produced islet-cell necrosis in rabbits while researching the nephrotoxicity of uric acid derivatives. Alloxan is a specific toxin that inactivates the pancreatic β cells, provoking a state of primary deficiency of insulin without affecting other islet types (Akah and Okafor, 1992). Hence, alloxan was selected to induce diabetes in the present study. Based on a preliminary study, batches B and C were used for the *in vivo* study. As shown in Figure 1, the decrease of glycemia started within the first few hours after oral administration of the extract. This lag time could be due to the time required for *V. amygdalina* extract to reach the site of the gastrointestinal tract where the active constituent of the extract could be absorbed. The glycemic profiles indicate that both doses of extract and the conventional tablet displayed similar biological activity. The hypoglycemic action of extract (150 and 300 mg) and the positive control demonstrated rapid onset time of 2 days reaching maximal activity within 12 days. No biological effect was seen in the group treated with distilled water (DW) alone, the slight reduction in the blood glucose level was due to the effect of long term glycemia. It is well known that prolonged glycemia can cause a slight decrease in blood glucose level (Ojiako and Nwanjo, 2006; Abdel-Barry et al., 1997). As illustrated in Figure 1, both 150 and 300 mg doses administered significantly decreased blood glucose levels ($P < 0.05$) compared to the non-treated group.

The real mechanism by which the extract performed its action was not clear. The results obtained from the present study clearly confirmed that the tested extract possesses marked hypoglycemic activity on the alloxan-induced diabetic and non-diabetic rats. From the present

experimental results, it could be suggested that, the extract exhibited dose dependent glucose lowering effects. The result of glucose lowering potentials of the extract is consistent with earlier reports on the hypoglycaemic action of the extracts of *V. amygdalina* in rats (Samy and Gopalakrishnakone, 2007). Nimemibo-Uadia (2003) attributed this action to tannins present in the extract of *V. amygdalina*, whereas other researchers presupposed a mechanism unrelated to insulin secretion from pancreatic β cell (Ebong et al., 2006). Be that as it may, it is probable that the two mechanisms may exist; one related to insulin production and the other targets peripheral carbohydrate metabolism. The former endows it with the ability to exert hypoglycaemia in diabetic rats, whereas the later achieves hypoglycaemia in non-diabetic rats. Alloxan is known to mediate pancreatic β -cells destruction via reactive oxygen species (ROS) generation (Szkudelsiki, 2001), depriving the animal of insulin, hence causing diabetes. *V. amygdalina* water extract having the ability to abate this alloxan-induced diabetes must necessarily have a corrective impact on the hitherto destroyed β -cells of the pancreas. It is possible to suppose that the antioxidant effect reported by Igile et al. (1994) to include luteolin, 7-O-beta glucuronoside and luelin, 7-O-betaglucoside may have attempted to reverse the cytotoxic effect of alloxan or at least to mop up the free radicals generated by alloxan responsible for beta cell destruction. By so doing, the β -cells could have started a gradual regeneration, hence insulin production commences to start an effectual control of hyperglycemia. On the other hand, the phytochemicals-endowed *V. amygdalina* may possess some alpha-glucosidase inhibitors as secondary plant metabolites. Such metabolites may competitively inhibit intestinal brush border enzymes-glucosidase, as well as pancreatic beta-amylase with the ultimate reduction in digestion and subsequent absorption of carbohydrates from the gut (Igile et al., 1994). Other researchers have proposed a parallel mechanism for tannins in their explanation (Igile et al., 1994). These were further strengthened by a researcher (Winleman, 1998), who indicated a strong positive correlation between the presence of flavonoids glycosides and phytosterols in plants and hypoglycaemic and antihyperglycemic activities, respectively. It is probable that the *V. amygdalina* extract may be endowed with both. In a related research by Ali et al. (1993), the author observed similar result in a different extract and the author resolved that the anti-diabetes effect was due to the alkaloid present in the plant.

Toxicological evaluation is an extremely important part of herbal formulation and development. High dose of extract is usually used for the study (Momoh et al., 2013). At the dose used for this evaluation, no toxic effect was observed on treatment with excess dose 20 to 30 times higher than the effective dose used in blood glucose level reduction. The physiological behavior of the rats remained

normal, there was no record of death and there was no sign of reduced activity after the administration of the 6,000 mg/kg, the response to stimuli (touch) were very good and sharp. The water and food intake were those not change compared to the negative control group. The oral LD₅₀ of the extract was estimated to be greater than 6,000 mg/kg body weight. Based on the result of this evaluation, we observed that this extract may be safe for human consumption. Pharmaceutical formulations for human consumption need to undergo safety evaluation in animal. This is a critical part of preclinical studies and forms an integral initial dossier during drug development. It is on these, that, clinical protocols, relevant instruction and contraindication and dosage are designed.

Hematological parameters are relevant to risk evaluation as the changes in haematological system have a higher predictive value for human toxicity, when data is translated from animal studies. The results in Table 4 indicated that there were significant changes ($P > 0.05$) in the haematological parameters of the treated groups compared to the diabetic and non-diabetic control and groups. Levels of white blood cells (WBC), packed cell volume (PCV) and haemoglobin (Hb) studies increased following repeated administration of different concentrations of the extract. The result indicated that the extracts was able to increase the secretion of the insulin which in turn halt gluconeogenesis and glycogenolysis which are serious factors in the loss of body weight in diabetic groups. The reversal of these pathways led to reduced glycosylation of the haemoglobin, hence the observed increase in the haematological parameters in the treated groups. Table 4 shows the results of the administration of graded doses (100, 150 and 300 mg) of extract of *V. amygdalina* on some liver function enzymes. The activities of both aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) increased in diabetic untreated group due to the liver damage which is associated with alloxan induced diabetes. The various concentrations of the extract (100 and 150 mg) significantly reduced the liver marker enzymes (AST, ALT and ALP) which was significant when compared with the control ($P > 0.05$). The decrease observed here is enough to encourage its use.

Contrarily, there was a slight increase in AST in the group that received 300 mg of the extract, although this can be overlooked because, alanine aminotransferase (ALT) is a more reliable marker of liver integrity than aspartate aminotransferase (Bassey et al., 1987). The observed slight increase in the activity of aspartate aminotransferase alone may be of extrahepatic origin due to the effect of the hyperglycaemia. It has been observed that the more specific cytosolic ALT, found in high concentration in the liver and AST, which is localized in the cytosol and mitochondria are released into the circulation and may not be completely due to the dose of the extract administered (Reitman and Frankel, 1997).

Conclusion

Our results confirm the traditional use of leaf extracts of *V. amygdalina* in treatment of diabetes mellitus. The dose of extract of *V. amygdalina* used in this study, was very effective and safe in treatment of hyperglycemic condition, and equally restored the imbalance in liver enzymes markers and hematological parameters associated with diabetes condition. Consequently, this plant extract could be used alone or in combination with other agents in the management of diabetes. More so, it could also be recommended as a food supplement.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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