Effect of Acute and Chronic Administration of Hot Water Extracts of *V. Amygdalina* on Some Metabolic Parameters in STZ-induced Hyperglycemic Rats

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Abstract. Medicinal plants are globally used in the management of diabetes mellitus, with some meeting up with scientific preconditional assessments by way of ascertaining the actual hypoglycemic effects of such plants. Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, often leading to severe complications if left unmanaged. The present study evaluates the effects of acute and chronic administration of hot water extract of Vernonia amygdalina (bitter leaf) (HWE-VA) on streptozotocin (STZ)-induced hyperglycemic rats. A total of 20 healthy male Wistar rats (205-251 g) were divided into two equal groups, viz control – administered with equi-volume of distilled water and Experimental – made severely hyperglycemic with streptozotocin (STZ) via the *intraperitoneal route* and administered with crude hot water extract of *V. amygdalina* (750 mg/kg). Experimental groups received either the extract or remained as hyperglycemic controls, with parameters including body weight, food intake, faecal output, water intake, and urine output monitored over time. Results demonstrated a significant increase in body weight, reduced food intake, and moderated water consumption in the extract-treated groups compared to hyperglycemic controls ($P<0.05$). These findings suggest that Vernonia amygdalina may possess antihyperglycemic properties, potentially improving metabolic parameters in diabetic conditions. Further research is needed to elucidate the mechanisms underlying these effects and assess their therapeutic potential for diabetes management.

Keywords: Hypoglycemia; Body weight; Food intake; Water intake; Faecal output; Urine output; Diabetes mellitus; *V. amygdalina*.

INTRODUCTION

The staple food in developing countries is mostly carbohydrates with challenges of poverty and paucity of health facilities with predisposing metabolic health challenges, including Diabetes mellitus (DM). Metabolic syndrome (MetS) represents a cluster of metabolic abnormalities that include hypertension, central obesity, insulin resistance, and atherogenic dyslipidemia and is strongly associated with an increased risk for developing diabetes and atherosclerotic and nonatherosclerotic cardiovascular disease (CVD) [1]. In 2020, about 3% of children and 5% of adolescents had metabolic syndrome, with some variation across countries and regions [2]. With the successful conquest of many of the old infectious diseases in the world, non-communicable diseases (NCD) have become the major cause of morbidity and mortality not only in the developed world but also in

underdeveloped countries. Among all these NCDs, metabolic syndrome has been the global scourge [3]. A deficiency of insulin secretion leads to increased blood glucose levels and organ damage, further disrupting the metabolism of the three major nutrients: lipids, carbohydrates, and proteins [4, 5]. It is estimated that there are approximately 250,000 higher plant species worldwide, out of which only a few have been screened for pharmacological activity according to their traditional use [6]. The American Diabetes Association (ADA) reported diabetes as an international public health issue.

Furthermore, the International Expert Committee recommended an alternative diagnostic index testing for diabetes using glycated (A1c) [7]. Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [8]. The widespread use of herbs throughout the globe has raised serious concerns over their quality, safety, and efficacy. As a result, exact scientific assessment has become a precondition to accepting herbal health claims [9]. *Vernonia amygdalina* is a perennial plant with a height between 1 m and 6 m, a widely grown shrub plant in Africa that is commonly consumed as a vegetable / as a culinary herb in soup and as food vegetable [10] and has high medicinal value due to its wide application in the treatment and management of various diseases with activities result of diverse bioactive compounds isolated from the different parts of the plant [11]. The biologically active compounds of *Vernonia amygdalina* are saponins and alkaloids [12], terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones and anthraquinone [13], edotides [14] and sesquiterpenes [15]. Different ethnic groups all over Africa have names for *V. amygdalina*. In Nigeria- Ewuro, Onugbu, Oriwo, Etidot, Ityuna, Chusar doki, and Fatefata [16] where some of the diverse medicinal uses of various parts of the plant and forms which it is used, including for breast milk enhancement with the dried leaves are powdered and taken orally; Malaria, where leaves and young stem and diabetes as Infusion [17–19].

The high number of children and adolescents living with metabolic syndrome globally highlights the urgent need for multisectoral interventions to reduce the global burden of metabolic syndrome and the conditions that lead to it, including childhood overweight and obesity [2]. All these palpable challenges encapsulated in the increasing poverty and paucity of healthcare facilities call for the exploration of cheaper and more natural and accessible means of Medicare in medicinal plants such as V. amygdalina, thus the aim of this study.

Figure 1 – Image of *V. Amygdalina* Plant [20]

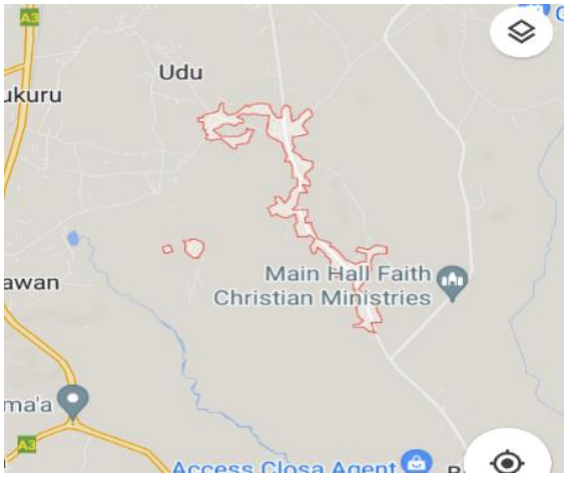

Figure 2 – Google map of Du District [21]

MATERIALS AND METHODS

Collection and Preparation of V. amygdalina Plant Material. A fresh plant sample of *V. Amygdalina* was collected in the early hours of the day and taken to the Federal College of Forestry Jos, Nigeria, where it was identified and authenticated. Having identified the plant sample, Fresh leaves of *V. Amygdalina* harvested from the same location of the collection were harvested, washed in clean water, and shade-dried in a well-ventilated area of the Pharmacology Laboratory of the College of Medicine and Allied Health Sciences, Bingham University, Jos Campus – Nigeria for 14 days. The dried leaves were pulverized into powder using a mortar, pestle, and a fine sieve. The powder samples were further stored in dark-airtight Ziploc plastic bags until required.

Hot Aqueous Crude Extraction of Powdered Sample of V. amygdalina. 100 g of the powdered leaves of *V. amygdalina* were weighed and loaded into different thimbles, and two different Soxhlet extractors were fixed with a lagged glass wool sidearm. The Soxhlet extractor and condenser were then fixed to a round bottom flask containing distilled water (1000 ml) placed on a six-channel heating mantle and adjusted for a hot water extraction temperature of 60 °C. On completion of the process, the extract was dried in a rotary evaporator, weighed, and stored at -4 °C in a refrigerator until use was according to the methods [22].

Experimental animal procurement and protocols. Twenty-five healthy male albino rats (Wistar strain), weighing between 205 and 251 g, were sourced from the Animal House of the Department of Pharmacology of the University of Jos, Nigeria. The rats were acclimatized for 7 days and maintained with pelleted feed, clean water and a conducive environment according to the required best practices [23].

Animal acclimatization and Induction of Hyperglycemia. The 20 animals were fasted overnight but with access to clean drinking water *ad libitum* and then made hyperglycemic by administration of 80mg/kg streptozotocin (STZ) used in 0.1 M sodium citrate buffer, pH4.5 via intraperitoneal (*i.p.)* route as a single dose. Each animal's Fasting glucose concentration (FBG) from the venous blood sample was measured using a One Touch Ultra glucometer (Accu-Check Active, Roche) after 24 hours to confirm sustained hyperglycemic status. The rats with BGC > 16.7 to 33.3 mmol/L (300–600 mg/dl) were considered hyperglycemic according to the method [22] but with slight modification. Thirty confirmed hyperglycemic rats were divided into two equal groups of 15 rats each, singly placed in metabolic cages for the acute and chronic treatment studies and allowed 48 hours and 3 days, respectively, to stabilize in the cage environment.

Acute treatment studies. Ten hyperglycemic rats were randomly allocated into two groups of 5 animals each. The rats were put singly in metabolic cages and allowed 48 hours to stabilize in the cage environment. They were then given a single dose of the extract as outlined below: Group I – Equi-volume of distilled water (DW); Group II – 750mg/kg HWE-VA. This was carried out for 5 day duration.

Chronic treatment studies. This was carried out for 28 days duration. Ten hyperglycemic rats were again randomly allocated into two groups, also placed individually in metabolic cages, allowing 3 days for acclimatization. From day zero, the animals were treated daily as outlined below: Group I – Equi-volume of distilled water (DW); Group II – 750 mg/kg HWE-VA.

Measured parameters treatment studies. The following parameters were measured and recorded daily for 5 and 28 days for the acute and chronic studies, respectively: Body weight (g), Food intake (g), Water intake (ml), Faecal output (g) and Urine output (ml) were measured for the duration of the study, while the changes in these parameters (expressed in appropriate units) were plotted against time and effects observed compared with those of the control groups.

Statistical analysis. Collected data from both acute and chronic studies were expressed as mean ± SEM, graph and tables, while the difference between means of treated and control groups was considered significant $P \leq 0.05$. The SPSS version 20 statistical package was used for analysis.

RESULTS AND DISCUSSION

Effect on Body Weight. Following acute treatment with a single large dose of HWE-VA (750 mg/kg) via the oral route, in the first five days, both the hyperglycemic control group (HGC) and the extract-treated group (HWE-VA) showed a gradual increase in body weight. However, the increase was more pronounced in the HGC group (Table 1).

Table 1 – Effect of Acute Administration of Hot Water Extract of *V. amygdalina* on Body weight of STZ-induced Hyperglycemic Rats

Time (days)	Mean Percentage change in Body weight (%)	
	Group I (HGC)	Group II (HWE-VA)
1	+0.36 ± 0.005	+0.28 ± 0.009
2	+0.66 ± 0.009	+0.51 ± 0.006
3	+0.95 ± 0.011	+0.68 ± 0.018
4	+0.10 ± 0.032	+0.75 ± 0.062
5	+1.23 ± 0.15	+0.94 ± 0.091

Notes: n = 5, HGC = Hyperglycemic-control group, HWE-VA = Hot water extract of *V. amygdalina*, +% Change = increase body weight, -% Change = decrease in body weight

Following daily treatment with HWE-VA (750 mg/kg) over 28 days (chronic study), the

percentage increase in body weight of the treated animals was consistently higher than that of the control animals (HGC group) (Table 2).

The weight gain in the extract-treated group was significantly higher (*P<0.05), indicating a potential protective or restorative effect against weight loss due to diabetes (Table 2).

Table 2 – Effect of Chronic Administration of Hot Water Extract of *V. amygdalina* on Body Weight of STZ-induced Hyperglycemic Rats

Time (days)	Mean Percentage change in Body weight (%)	
	Group I (HGC)	Group II (HWE-VA)
2	0.4 ± 0.02	1.4 ± 0.09
4	1.2 ± 0.018	2.4 ± 0.17
6	1.3 ± 0.078	3.6 ± 0.47
8	2.0 ± 0.016	4.3 ± 0.16
10	2.6 ± 0.014	5.8 ± 0.3
12	3.5 ± 0.02	6.3 ± 0.92
14	3.9 ± 0.14	7.4 ± 0.84
16	4.8 ± 0.24	8.1 ± 0.74
18	5.3 ± 0.12	9.1 ± 1.05
20	5.9 ± 0.53	10.0 ± 0.75
22	6.8± 0.61	10.7 ± 1.02*
24	7.6 ± 0.52	11.8 ± 1.8*
26	8.3 ± 0.94	12.2 ± 1.92
28	9.2 ± 1.2	12.3 ± 2.1

Notes: *P<0.05 versus corresponding control, n = 5, HGC = Hyperglycemic-control group, HWE-VA = Hot water extract of *V. amygdalina*

In diabetes, loss in body weight is a common phenomenon. This is characterized by increased appetite insulin deficit that reduces all anabolic processes and increases catabolic processes, resulting in further body weight loss [25]. Moreover, authors [26] reported that a decrease in body weight is thought to be due to the catabolism of fats and proteins in the tissues caused by an insulin deficiency. The steady increase in body weight with time in the rats administered with chronic HWE-VA (750 mg/kg) suggests its hypoglycemic potential in the STZ-induced hyperglycemic group compared to the control group. The body weight gain is significant on days 22 (10.7 ± 1.02) and 24 (11.8 ± 1.8). The present study aligns with reports using the same model [27, 28]. The ability of V. amygdalina to promote weight gain suggests a possible protective effect against diabetes-induced weight loss, which is a common symptom of uncontrolled diabetes.

Effect on Food Intake and Fecal Output. Table 3 shows a sudden but transient decrease in food intake and faecal output immediately after acute administration of HWE-VA (750 mg/kg).

Table 3 – Effect of Acute Administration of Hot Water Extract of *V. amygdalina* on Food Intake and Faecal output of STZ-induced Hyperglycemic Rats

Time (days)	Group I (Mean HGC)		Group II (Mean HWE-VA)	
	Food Intake (g)	Faecal output (g)	Food Intake (g)	Faecal output (g)
1	3.1 ± 0.07	2.4 ± 0.09	3.6 ± 0.29	2.5 ± 0.69
2	3.7 ± 0.02	3.0 ± 0.06	2.1± 0.17*	1.0 ± 0.71*
3	4.6 ± 0.05	3.3 ± 0.03	2.9± 0.42*	1.9 ± 0.43*
4	4.9 ± 0.11	3.8 ± 0.07	3.1 ± 0.66*	2.1± 0.56*
5	5.3± 0.1	4.0 ± 0.16	3.4 ± 0.78*	2.4± 0.45*

Notes: *P<0.05 versus corresponding control, n = 5, HGC = Hyperglycemic-control group, HWE-VA = Hot water extract of *V. amygdalina*

The extract-treated group had significantly lower food intake and faecal output from day 2 onward than the control (*P<0.05). The reason for this decrease (though transient) is in tandem with [28], who reported that the increase in food consumption could be explained either by the decrease in the activity of the leptin receptor in the hypothalamus following an insulin deficiency or by the decrease in the release of hormones promoting satiety (cholecystokinin, peptide YY and glucagon-like peptide-1) [24].

For chronic administration with HWE-VA (750 mg/kg) over 30 days, food intake in the extract-treated group remained consistently lower than the control group, with significant differences noted from day 20 onward. Similarly, faecal output was slightly lower in the treated group, but the differences were statistically significant only at certain time points (Table 4).

This reduction in food intake suggests that *V. amygdalina* may help control excessive appetite (polyphagia), which is commonly observed in diabetic conditions.

Table 4 – Effect of Chronic Administration of Hot Water Extract of *V. amygdalina* on Food Intake and Faecal Output of STZ-induced Hyperglycemic Rats

Time (days)	Group I (Mean HGC)		Group II (Mean HWE-VA)	
	Food Intake (g)	Faecal output (g)	Food Intake (g)	Faecal output (g)
0	3.1 ± 0.07	2.5± 0.09	3.4 ± 0.2	1.7± 0.09
5	4.05 ± 0.05	3.2± 0.16	3.8± 0.41	2.2 ± 0.3
10	4.7 ± 0.14	4.05± 0.41	4.3± 0.82	2.9 ± 0.35
15	5.7 ± 0.11	4.55 ± 0.69	4.2 ± 0.9	3.4± 0.42
20	6.2± 0.82	4.7± 0.18	4.7 ± 0.78*	3.5 ± 0.13
25	7.1 ± 0.46	5.0± 0.28	4.5 ± 0.99*	3.3± 0.34*
30	7.9 ± 1.12	5.1± 0.61	4.8 ± 0.51*	3.5± 0.47

Notes: *P<0.05 versus corresponding control, n = 5, HGC = Hyperglycemic-control group, HWE-VA = Hot water extract of *V. amygdalina*

Effect on Water Intake and Urine Output. Regarding acute administration of HWE-VA to STZ-induced Hyperglycemic Rats and the HGC group, water intake and urine output were slightly lower in the extract-treated group compared to the control, with significant reductions observed from day 4 (Table 5).

Table 5 – Effect of Acute Administration of Hot Aqueous Extract of *V. amygdalina* on Water Intake and Urine Output of STZ-induced Hyperglycemic Rats

Time (days)	Group I (Mean HGC)		Group II (Mean HWE-VA)	
	Water Intake (ml)	Urine Output (ml)	Water Intake (ml)	Urine Output (ml)
1	2.4 ± 0.41	2.2± 0.49	4.0 ± 0.92	03.4 ± 0.98
2	8.0 ± 0.98	4.4± 0.86	6.6± 0.98	5.7 ± 0.86
3	11.2 ± 1.02	6.8± 0.97	9.6 ± 0.42	8.3± 0.42
4	14.4 ± 1.66	8.3 ± 1.08	13.2 ± 1.15	11.0 ± 1.06*
5	17.9. ± 2.06	10.1± 1.14	14.8 ± 1.03*	12.7± 1.72*

Notes: *P<0.05 versus corresponding control, n = 5, HGC = Hyperglycemic-control group, HWE-VA = Hot water extract of *V. amygdalina*

Chronic administration: Over 30 days, the HWE-VA-treated group had significantly lower water intake and urine output (*P<0.05), suggesting that bitter leaf might help reduce excessive thirst (polydipsia) and frequent urination (polyuria), both of which are symptoms of diabetes (Table 6).

Table 6 – Effect of Chronic Administration of Hot Aqueous Extract of *V. amygdalina* on Water Intake and Urine Output of STZ-induced Hyperglycemic Rats

Time (days)	Group I (Mean HGC)		Group II (Mean HWE-VA)	
	Water Intake (ml)	Urine Output (ml)	Water Intake (ml)	Urine Output (ml)
0	8.2 ± 0.64	0	6.2 ± 0.64	0
5	11.8 ± 0.92	2± 0.11	9.8± 0.8	2.6 ± 0.09
10	14.1 ± 1.02	3.6± 0.34	13.4 ± 0.99	5.6 ± 0.16
15	16.6 ± 1.86	5.3 ± 0.65	15.0 ± 1.82	6.6± 0.26
20	19.1 ± 1.90	6.5± 0.46	17.2 ± 1.09	7.5 ± 0.74
25	20 ± 1.03	7.5± 0.92	17.5 ± 1.63*	9.0± 0.97
30	20.4 ± 2.04	8.2 ± 0.64	18.1 ± 2.09*	9.5± 0.46

Notes: *P<0.05 versus corresponding control, n = 5, HGC = Hyperglycemic-control group, HWE-VA = Hot water extract of *V. amygdalina*

Having induced the experimental rats with hyperglycemia using STZ, which should be characterized by an increase in water intake with consequent excessive elimination of urine volume with loss of glucose, the consistent increase in the urine output by the chronic administration of HWE-VA (750 mg/kg) further suggests it's hypoglycemic effect on the STZ-induced hyperglycemic group. Authors [29] also reported the three main features of Type 2 Diabetes Mellitus: weight loss, polyphagia, and polyuria. In the present study, these features were significantly controlled in the chronic compared to the acute administration of HWE-VA (750 mg/kg). Various reports on the phytochemicals/metabolites found in

V. amygdalina support the observed hypoglycemic effect of HWE-VA (750 mg/kg) administered to *the* STZ-induced hyperglycemic group in the present study. These reports includes the revelation of the following flavonoids: apigetrin, apigenin, luteolin, diosmetin, baicalin, rhoifolin, and scutellarin via LC-MS/MS analysis to investigate the phytochemicals found in *V. amygdalina* [30], the presence of vernonioside A3, vernodalol, vernolepin, vernodalin, 11,13-dihydrovernodalin, and hydroxyvernolide among the isolated bioactive chemicals and flavonoids found in *V. amygdalina* [31] and bioactive compounds extracted from *V. amygdalina* includes 6β,10β,14β trimethylheptadecan-15 α-olyl-15-O-β-D-glucopyranosyl-1,5 β olide, glucuronolactone, 11 α-hydroxyurs-5,12-dien-28-oic acid-3 α,25-olide, 10-geranilanyl-O-β-D-xyloside, 1-heneicosenol O-β-D-glucopyranoside, apigenin, luteolin (3',4',5,7tetrahydroxyflavone), vernolide, hydroxyvernolide, 3′-deoxyvernodalol , vernodalol, diterpene (ingenol-3-angelate), vernomygdin, 4-methylumbelliferone, cephantharin, cryptolepine, isocryptolepine, neocryptolepine, courmarins, vernolepin, and vernoniosides [32], also in tandem with [33] that asserted that flavonoid compounds may exert antidiabetic and antioxidant properties. The reduced water intake and urine output in this study suggest that V. amygdalina may alleviate polydipsia (excessive thirst) and polyuria (frequent urination), which are hallmarks of diabetes.

CONCLUSIONS

Hot water extract of *V. amygdalina* (HWE-VA) had a hypoglycemic effect indicated by stabilizing the metabolic parameters (body weight, food intake, water intake, faecal output and urine output) of STZ-induced hyperglycemic rats by preventing excessive weight loss, reduced food intake, indicating appetite-regulating properties and creased water intake and urine output in the animals significantly, thus improving glucose tolerance and suggesting that it possess antidiabetic property. The promising chronic administration results indicate that sustained extract use might help mitigate diabetes-related metabolic disturbances. These findings support the traditional use of *V. amygdalina* in diabetes management. Further studies are recommended to explore its long-term effects and mechanisms of action.

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