

Evaluation of Activities of Bitterleaf (*Vernonia Amygdalina*) Extract on Haemostatic and Biochemical Profile of Induced Male Diabetic Albino Rats.

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Abstract: The activities of Bitter leaf (*Vernonia amygdalina*) aqueous extract on the haemostatic, haematological and biochemical profile of induced male diabetic albino rats were investigated. Twenty one (21) male albino rats (7-8 months) within the weight range of 130-150g were used. They were placed into three groups each consisting of seven rats. Group 1 (control), Group 2 (induced diabetic rat on bitter leaf extract).and Group 3 (Induced diabetic rats on dianil tabs). They were kept in iron cage with constant light source (12 hrs per day) in animal house and for two weeks with normal rat feed. 1.0ml of the extract was administered daily to each of the rat using blunt syringe for 28 days in addition to their rat feed. The control group received clean water instead of the extract. 2.0ml of blood sample were collected via cardiac puncture from both the test rats and the control rats for analysis. The result obtained in the haemoglobin level estimation and packed cell volume did not indicate much significant difference ($p < 0.05$). The haemoglobin estimation for control rat is 12.8 ± 0.75 g/dl, and test rats 12.57 ± 0.46 (Group 2) and 13.1 ± 0.6 g/dl(Group3). On fasting blood sugar, the value obtained in control rats was 85.5 ± 1.0 g/dl and in test rats 68 ± 0.7 (group 2) was 64 ± 2.8 mg/dl in (group 3) respectively showing a significant difference ($p < 0.05$). the value obtained in random blood sugar level in control rats (group 1) was 94 ± 8.63 mg/dl in control rats, 82.3 ± 3.4 mg/dl (group 2) and 80.0 ± 6.0 mg/dl (group 3) also indicating a significant difference ($p < 0.05$), in packed cell volume, the value obtained were 37.0 ± 1.2010 (control), $36.0 \pm 1.2\%$ (group 2) and $34.0 \pm 4.2\%$ (group 3) $p < 0.05$ (not significant).

On urine output, the control rats (Ggroup1) was 4.76 ± 0.45 ml, while the test rats on aqueous extract feed of veronica amydalina was 3.15 ± 0.3 ml and those on dianil was 2.3 ± 0.3 ml respectivel. It can be deduced that *Vernonia amydalina* contains some principles that maybe interfering with insulin production and metabolism causing reduction in glucose level of albino wistar rats. The bleeding and clotting times, Prothrombin times were shortened ($p < 0.05$) indicating interference in haemostatic mechanism of albino rats.

Short Running Title: Biochemical, diabetic rats, bleeding times, clotting times.

I. Introduction

Vernonia amygdalina commonly called "Bitter leaf" is a medium sized shrub with petiolate green leaf of about 6mm diameter and elliptic in shape. This leaves have found relevance in traditional folk medicine as anti helmintics, anti malaria, anti microbial, anti cancer and as a laxative herb. This study is designed to evaluate the activity of this herbaceous plant on haemostatic mechanism and biochemical profile of induced diabetic male albino rats. The genus *vernonia* is named by English botanist William Vernon. Several species of *vernonia* including *V.calvoana*, *V.amygdalina*, and *V.colorata* are eaten as leaf vegetables (Akah and Okafor1992). Common names for these species include onugbu(Igbo), Ndole (cameroun), Ewuro(Yoruba), Shawaka(Hausa) Oguntola 2013. They are common in most west African and central African countries. The leaves have a sweet and bitter taste and are solid, fresh or dry, and are a typical ingredient in melon(Egusi)soup. The leave can be eaten like spinach in soup or dried too. Diabetes mellitus (DM) is a common disorder associated with increased morbidity and mortality and can be regarded as a group of metabolic diseases characterized by chronic hyperglycemia due to effective insulin secretion , insulin action or both resulting impaired carbohydrate, lipid, protein metabolism. The use of *vernonia amygdalina* in the treatment of diabetes mellitus is very common among ethno medical practitioners, hence this research is also aimed at authenticating the use of this herb in treating diabetes by herbalist. Fluids from fresh leaves are pressed out and applied to bleeding cuts and wound and will stop the bleeding of minor wounds (Oguntola, 2013), thus exhibiting a haemostatic property.

Other medicinal value of bitter leaf:

Regular consumption of vegetables such as *Vernonia amygdalina* (Bitter leaf) and *Telfaria occidentalis* (Ugu) can help to regulate blood cholesterol level, a risk factor for heart attack and stroke.

This build up of cholesterol and other substances called plaque, can narrow the artery like a clogged drain, leading to arteriosclerosis, or hardening of the arteries over time, this causes heart attack (Oguntola, 2013).

The 2011 study published in African Journal of Biochemical Research showed that the treatment with bitter leaf and ugu diets led to a significant increase in serum good cholesterol (HDL-C) showing their protective role in condition that affects the heart and blood vessels such as heart attack.

Also common skin diseases such as ringworm, eczema and others have been successfully treated with bitter leaf because of its antimicrobial effect. In many communities, individuals squeeze and paste bitter leaf regularly on the affected portion of the skin, coupled with the drinking of the fresh bitter leaf juice. This clears the skin diseases in short time.

II. Materials and methods: Animals:

Twenty one (21) male albino rats were randomly selected. They were kept in animal house for 14 days to get acclimatized to the environment before commencing an acute feeding which lasted for 30 days. The rats were housed in a wire mesh cage under standard conditions (Temperature 25-30°C, 12hr light and 12hr darkness cycle). They were allowed free access to water and feed produced by Pfizer, Nigeria limited Benin Edo state), throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use by animals.

Plants materials:

Fresh leaves of *V. amygdalina* were obtained freshly from a farm in Umuoma Uli, and the leaves were identified by a taxonomist being used for the research study.

Experimental design:

Twenty-one (21) male albino rats weighing 130-150g were placed into three groups namely Group 1 (control) Group 2 (induced diabetic rat on bitter leaf extract) and Group 3 (induced diabetic rats on dianil tablets). The test rats were fed on normal rat pellet and water ad libitum in addition to oral administration of 5mg/ml once daily of the aqueous leaf extract of *V. amygdalina*.

PREPARATION OF EXTRACT OF *VERNONIA AMYGDALINA*:

Fresh leaves of *V. amygdalina* were collected, washed very well to remove saponins to concentrations that are okay for the body to handle; they were weighed, cut and left to dry in hot air oven.

The crude extraction method by Ugochukwu et al 2003, and Edet et al (2009) was used for the preparation of the extract. The leaves were further dried in sunlight for 18 days. The dried sample was crushed to powder using a mechanical blender. The powdered leaves were stored in a glass bottle with a plastic screw cap and kept in a refrigerator (4°C). The leaves were homogenized with distilled water by shaking and kept for 12 hours. The mixtures were filtered with Whatman No. 1 Filter paper. The filtrates were concentrated to one tenth (1/10) of the original volumes at 38-40°C using a rotary evaporator. The aqueous extract was freeze dried yielding a brown powder. The powders were resuspended in distilled water to make a concentration of 5mg/ml before use. The extract was administered to the rats using a 5ml syringe with blunt needle.

PHYTOCHEMICAL ANALYSIS OF THE LEAVES:

The aqueous extract of the leaves was screened for the presence or absence of various secondary metabolites using standard phytochemical screening procedures as described by Harbourne (1973) and Trease and Evans (1996).

The extract was tested for resins, calcium, alkaloids, flavenoids, reducing sugars, saponins, glycosides, carbohydrates, steroids, acidic compounds, fats and oils.

TOXICITY STUDIES:

The LD₅₀ of extracts in albino rats was determined using Lorke's method (1983). The procedure of determining the lethal dose is by increasing the concentration of the extracts administered to the rats (per body weight) in each group consisting of eight (8) rats per group for five (5) days. The concentration used are 500mg/kg, 100mg/kg, 2000mg/kg, 3000mg/kg, 4000mg/kg, 5000mg/kg, 6000mg/kg, and 7000mg/kg respectively. The mortality rate was determined and a graph plotted to determine the LD₅₀.

INDUCTION OF DIABETES (HYPERTHYCAEMIA) IN RATS:

Hyperglycemia (diabetes) was induced in male wister albino rats by intraperitoneal administration of alloxan monohydrates (150mg/kg body weight) dissolved in normal saline given at the rate of 0.4ml at 48 hours intervals. The rats were fasted for 18 hours before the first injection. The alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 30% glucose solution orally at different time intervals after 6 hours of alloxan induction of 5% glucose solution was kept in bottles in their cages for the next 24 hours to prevent hypoglycemia.

After 7 days, the rats with diabetes mellitus glycosuria (indicated by urinalysis and blood glucose estimation), and hyperglycemia with glucose range of 300-350mg/dl were used for the tests. Induction of diabetes was accomplished by three doses of alloxan that was administered at third injection, the rats were fasted again and the blood glucose level was recorded.

ORAL ADMINISTRATION OF THE AQUEOUS EXTRACT:

After the induction of diabetes in the rats, all the induced rats with their blood sugar levels from the range of 300-350mg/dl were subjected to feeding with the aqueous extract of bitter leaf for 28 days. The level of the needle (i.e. the sharp edge was cut off to make it blunt). The volume forced into the mouth of the animal is 2.0ml of the extract. They were allowed access to their normal rat feed and drinking water ad libitum.

COLLECTION OF SAMPLES FOR TESTS:

At the end the acute feeding (28 days) of the extracts, cardiac puncture was used to collect 2.0ml of blood samples from the diabetic rats and control rats into EDTA bottles for tests. Their weight, full count blood count including platelet count, bleeding time, clotting time and prothrombin time were measured.

DETERMINATION OF HAEMOSTATIC AND BIOCHEMICAL PROFILE:

Blood samples collected into EDTA sequestrene bottles were used for the haematological studies within hours of sample collection while those collected in sodium citrate bottle were spun and the plasma extracted for the determination of haemostatic profile. The packed cell volume was determined according to the haematocrit method described by Alexander and Griffiths 1993, while the white blood cell count and platelet count were determined according to the visual method of Dacie and Lewis (1991).

The whole blood clotting time and the bleeding time were carried out by the method of Dejana et al, 1982. The prothrombin time test was carried out by the method of Quick's one stage method as described in Baker (1985). The levels of blood sugar was determined using B.G meter.

STATISTICAL ANALYSIS:

The data obtained from the study were expressed as mean and standard deviation (Mean ± S.D) while student's t test was used to compare the result of the control and the test. A P-value of less than or equivalent to (p<0.05) or (p=0.05) was regarded as statistically significant.

III. Results:

Table 1: The phytochemical analysis of *Vernonia amygdalina* (Bitter leaf).

| | CONSTITUENTS IN EXTRACT OF BITTERLEAF | | | | | | |
|-------------------------|---------------------------------------|-------------------|---------|--------|------------|----------|---|
| | Alkaloids | Acidic Compounds. | Calcium | Tanins | Falvenoids | Saponins | Steroids Fats and oils Resins Terpenoids Glycosides Reducing sugar |
| Degree of Concentration | +++ | + | ++ | + | + | ++ | - |

- Negative (absent)
- + Present in small concentrations
- ++ Present in moderately high concentrations
- +++ Present in very high concentrations.

TABLE 2:Haematological profile of control rats and those gavaged with water extracts of *V. amygdalina* for 30 days ($p < 0.05$).

| Extracts | Hbg/dl \pm S.D | PCV% \pm S.D | WBC/mm ³ \pm S.D | Platelets x 10 ⁹ /l \pm S.D | P-value |
|---|------------------|----------------|-------------------------------|--|------------|
| Group 1 (control) n=7 (Extract free) | 12.8 \pm 0.76 | 37 \pm 1.2 | 4,120 \pm 522 | 160 \pm 38 | - |
| Test Rats | | | | | |
| Group 2: Day 1 before induction of Diabetes n=7 | 12.5 \pm 0.8 | 37 \pm 2.4 | 4,400 \pm 318 | 162 \pm 35 | - |
| Group 2: Diabetic rats 30 days after extract feed | 12.48 \pm 0.4 | 36.0 \pm 1.2 | 4,100 \pm 492 | 168 \pm 28 | $p > 0.05$ |
| Group 3: Day 1 before induction n=7 | 11.62 \pm 1.4 | 34.0 \pm 4.2 | 3,800 \pm 236 | 159 \pm 16 | - |
| Group 3: Diabetic rats. 5 days after dianil tabs | 11.60 \pm 0.8 | 33.1 \pm 2.4 | 3,750 \pm 230 | 157 \pm 41 | $p > 0.05$ |

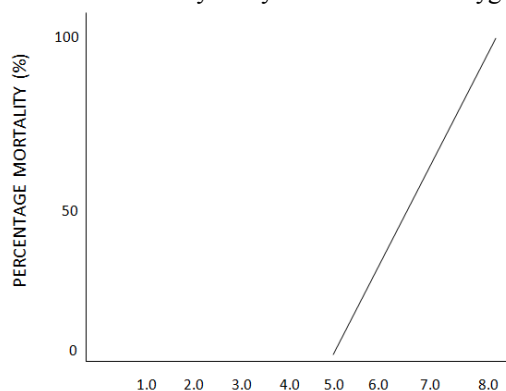
TABLE 3: Effects of extract of *V. amygdalina* on haemostatic mechanisms of male albino rats ($p < 0.05$).

| Extracts | Bleeding time \pm S.D | Clotting time mins \pm S.D | Prothrombin Time Sec \pm S.D | P-value |
|--|-------------------------|------------------------------|--------------------------------|------------|
| Group1 n=7 (control extract free) | 4.6 0.5 | 5.2 \pm 0.9 | 13.0 \pm 0.6 | - |
| Test rats Group 2, n=7. Day 1 before Diabetes induction | 2.4 \pm 0.7 | 5.6 \pm 0.7 | 14.5 \pm 0.4 | - |
| Group 2 Diabetic rats 30 days after <i>V. amygdalina</i> extract | 1.3 \pm 0.4 | 2.6 \pm 0.3 | 10.5 \pm 0.2 | $P < 0.05$ |
| Group 3 n=7 day 1 before diabetes induction | 2.4 \pm 0.8 | 5.8 \pm 1.3 | 14.2 \pm 0.5 | - |
| Group 3 n=7 diabetic rats 5 days after dianil tabs | 2.4 \pm 0.7 | 5.7 \pm 1.0 | 14.0 \pm 0.2 | $p > 0.05$ |

Table 4: Biochemical profile and urine volume of male albino rats on water extract feed of *V. amygdalina* for 30 days.

| Extracts | FBS mg/dl \pm S.D | RBS mg/dl \pm S.D | Urine volume ml \pm S.D | P-value |
|--|---------------------|---------------------|---------------------------|------------|
| Group 1 (control) Extract free | 85 \pm 1.0 | 94.0 \pm 8.6 | 4.86 \pm 0.96 | - |
| Test rats Group 2, n=7 Day 1, before diabetes induction | 88 \pm 2.2 | 103 \pm 2.3 | 4.76 \pm 0.45 | - |
| Group 2, n=7 diabetic rats 30 days after <i>V. amygdalina</i> extracts | 68 \pm 0.7 | 82 \pm 3.0 | 3.15 \pm 0.3 | $P < 0.05$ |
| Group 3, n=7 Day 1 before diabetes induction | 86 \pm 3.5 | 96.6 \pm 3.4 | 4.80 \pm 0.25 | - |
| Group 3 n=7 Diabetic rats 5 days after dianil reference tab | 74 \pm 2.8 | 80 \pm 6.0 | 2.3 \pm 0.3 | $P < 0.05$ |

FIGURE 1: Lethality study of extract of *V. amygdalina*.



Log-Concentration of extract (mg/dl).

IV. Discussions

The activities of Bitter leaf (*Vernonia amygdalina*) extract on haemostatic and biochemical profile of induced male diabetic albino rats have been evaluated. Most times, plant extracts are given to humans in excessive doses by the ethnomedical practitioners. Toxicity may affect the result of the study and so acute toxicity studies using LD₅₀ were carried out before the extract was administered to the animals. The result of lethality studies showed that the LD₅₀ in rats using Bitter leaf extract was 5000mg/kg (Fig 1). The volume (1ml) containing 5mg/kg concentration of the extract was far below the lethal dose and so was considered safe to the animal used throughout the period of study.

The activities of Bitter leaf extracts on haemostatic and biochemical profile provides physiological information on a blood assessment in the body (Ita et al, 2007). It is clearly shown in Table 2 that the extracts of *V. amygdalina* has no effect on haemoglobin concentration, packed cell volume, white blood cells and platelet count (p>0.05).

The result from the biochemical study showed that the tested aqueous leaf extract of *Vernonia amygdalina* induced significant reductions in the blood glucose concentrations of normoglycaemic and alloxan-induced diabetic rats. The findings of this investigation may therefore suggest that the plant extract could at least in part, stimulate insulin production and glucose utilization, like chlorpropamide to bring its hypoglycaemic effect in the mammalian experimental animals used. Although the present findings suggest the presence of hypoglycaemic compounds in leaf extract of *Vernonia amygdalina*, the precise mechanism of its hypoglycaemic action is still speculative and requires further studies for appropriation elucidation. It is however interesting to note that the plant extract, like chlorpropamide is as effective in reducing the blood glucose concentrations of diabetic rats as in reducing the blood glucose concentration of normoglycaemic rats.

Bitter leaf does not only reduce the blood sugar level drastically, it also helps to repair the pancreas (Ogundotola, 2013). According to a study published in the Journal of Pharmacy and Bioresources researchers at the University of Jos, Nigeria stated that the crude chloroform extract of the leaves of Bitter leaf has an antidiabetic effect in rats with diabetes mellitus (Type 2 Diabetes) under laboratory conduction.

The extract demonstrated haemostatic activity by shortening bleeding time, bleeding time and prothrombin time. Oguntola 2013 pointed out that the leaves of *V. amygdalina* if squeezed and placed on cuts would stop bleeding of injured vessels. It is rich in calcium, vitamin C and saponins. It was observed that calcium was a principal active ingredients present in plasma and bones. It is free calcium ions that are physiologically active in coagulation mechanism. Calcium ions are essential for the conversion of prothrombin to thrombin and for the normal action of heart muscle and for neuromuscular conduction.

References

- [1]. AKAH P.A and OKAFOR C.I (1992). Hypoglycaemic effect of *Vernonia amygdalina* in experimental rabbits. *Plant Med. Res.* 1:6-10.
- [2]. ALEXANDER R.R and GRIFITHS J.M (1993). Haematocrit in Basic biochemical methods 2nd Ed. John Willey and sons inc. Publication New York Pp 186-187.
- [3]. BAKER F.J (1985). Investigation for haemostatic abnormalities. Introduction for haemostatic abnormalities. Introduction to Medical laboratory Technology. Butterworths publications, Woburn London p 343.
- [4]. BG METER and STRIPS manufactured in Taiwan. Manufactured for FGICO (First Gulf International inc). 3055 Sage Road, No 210 Houston Texas 77056 USA. www.fgico.com.
- [5]. DACIE J.R and LEWIS S.M (1991). Practical haematology London. Churchill Living stone Pp 566.
- [6]. DEJANA E, DEGAETANO G (1982). Bleeding time in rats. A comparison of different experimental conditions. *Thromb. Haemostat* 48:108-111.
- [7]. EDET E.E, AKPANABIATU M.I, E no A.E, UMOH I.B, ITAM E.H (2009). Effect of Gongronema latifolium crude leaf extract on some cardiac enzymes of alloxan- induction induced rats. *African Journal of Biochemistry* 3(11)366-369.
- [8]. HARBOURNE J.B.C (1973). Photochemical method, Chapman and Hall. London p279.
- [9]. ITA S.O, ETIM O.E, BEN E.E, EKPO O.F (2007). Haematopoietic properties of ethanolic leaf extract of *Ageratum Conyzoides* (Goat weed) in albino rats. *Nigerian Journal of physiological sciences* 22(1-2)83-87.
- [10]. LORKE D, (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol* 54:275-278
- [11]. OGUNTOLA S, (2013). Things Bitter leaf can do Nigerian Tribune. [Com.ng/news.2013/index-----/15271.Things-bitter-leaf-can do.html](http://Com.ng/news.2013/index-----/15271.Things-bitter-leaf-can-do.html).
- [12]. TREASE G.E, EVANS W.C (1996).Textbook of Pharmacognosy, 14th Ed. W.B Saunders, London p.11.
- [13]. UGO N.H, BABADY N.E, COBOURNE M, GUSSET S.R (2003). The effect of Gongronema latifolium extract on serum lipid profile and oxidative stress in heatolytes of diabetic rats. *Journal of Bioscience* 28 (1) 1-5.