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EFFECTS OF ETHANOL LEAF EXTRACT OF VERNONIA AMYGDALINA D (BITTER LEAF) ON HAEMATOLOGY AND LIPID PROFILE OF APPARENTLY HEALTHY ALBINO RATS

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Abstract

Bitter leaf (*Vernonia amygdalina* Delile), family Asteraceae or Compositae is a perennial shrub that is widely used locally in Nigeria for both therapeutic and nutritional purposes. This study was conducted to evaluate the phytochemical composition and the effect of ethanol leaf extract of the plant on haematological parameter and lipid profile of rat. Sixteen (16) albino wistar rats were randomly divided into four groups of 4 rats each. All groups were fed with growers mash. Groups 2, 3 and 4 further received daily oral doses of 200 mg, 300 mg and 400 mg per kg body weight of the extract, respectively. After 14 days of administration, the animals were sacrificed and blood was collected through orbital-plexus. The presence of alkaloids, tannins, phenolics, saponins, flavonoids, terpenoids, glycosides, carbohydrate and resins were detected with saponins as the most abundant while steroids was found to be absent. Results equally showed a non-significant ($p>0.05$) decrease at the highest concentration (400 mg/kg body weight) in PCV (27.2± 1.7), Hb (75.30 ± 1.10), RBC (0.52± 0.01) and PLT (142.50 ±1.30). However, WBC (8.58± 0.50), MCH (153.69±2.80), MCV (0.41±0.0) and MCHC (332.0±2.90) increase significantly ($p<0.05$) at 400mg/kg when compare to control (41.0±1.8), (135.80±2.50), (1.31±0.03), (303.80±5.10), (1.47±0.40), (102.99±1.40), (0.33± 0.30) and (315.80±1.70) respectively. Result also indicated a non-significant ($p>0.05$) decrease in the lipid profile of the test rats relative to control. It was therefore concluded that the bitter leaf extract is rich in bioactive compounds but negatively affect the haematological parameters at 400mg/kg body weight with no remarkable effect on the lipid profile at the administered doses within the period of the research.

Keywords: Bioactive compounds, Bitter leaf, Haematological parameters, Lipid profile, *Vernonia amygdalina* Delile.

I. INTRODUCTION

From earliest times, plants have provided man with diverse means of healing (Kadiri, 2020). The medicinal value of these plants lies in some chemical substances that produce definite physiological actions in the human body (Udochukwu *et al*., 2015). Examples of these most important bioactive constituents are anthraquinones, flavonoids, saponins, polyphenols, tannins and alkaloids (Sofowora, 2006; Erasto *et al.*, 2006). These plants have been reportedly used in the treatment of ailments such as diabetes mellitus, breast cancer, hypertension, stomach disorder, fever symptoms and cough traditionaliy (Pinto and Rivlin, 1999; Li and Schellhorn, 2007; Odugbemi, 2006). One of such plants suspected to have these medicinal values is the bitter leaf. Bitter leaf (*Vernonia amygdalina* Delile) belongs to the family *Compositae* or *Asteraceae*. The plant is known as "Ewuro" by the Yorubas, "olugbu" by the Igbos and *"*shuwakaa*"*by the Hausas. It is a widely used local plant in Nigeria for both therapeutic and nutritional purposes. The leaves are green in colouration with a characteristic odour and bitter taste (Ijeh and Ejike, 2011; Akpaso *et al.*, 2011). The leaves of bitter leaf are used as soup condiments after washing and boiling to get rid of the bitter taste (Hamzah *et al*., 2013). It is popularly called bitter leaf because of its abundant bitter principles (Ekpo *et al.*, 2007). The bitter taste is due to anti-nutritional factors such as alkaloids, saponins tannins and glycosides (Butter and Bailey, 1973). The macerated leaves of the plants are consumed as vegetables and condiments (Arhoghro *et al*., 2009) while the water extract serves as tonic for the prevention of certain illnesses (Joseph *et al.*, 2013). Extract from the leaf has been shown to provide protection to the livers of rats pretreated with it prior to intoxication with acetaminophen overdose (Enemali and Udedi 2018).

This study, is therefore designed to determine the effect of graded concentrations of ethanol leaf extract of this all important plant on the haematology and lipid profile of rats after two weeks of administered.

Fig.1: Fresh Growing *Vernonia amygdalina* Plant.

II. MATERIALS AND METHODS

MATERIALS.

Plant Materials

The plant used for this study is the leaf of *Vernonia amygdalina* (bitter leaf) which was obtained from sarkin tila farm at tila village, keffi local Government Area of Nasarawa state, Nigeria.

Animals

Male Albino Rats were purchased from the Nigerian Institute of Veterinary Research (NIVR) Vom, near Jos, Plateau State.

METHODS

Collection and preparation of plant materials

Fresh leaves of bitter leaf were obtained from sarkin tila farm at tila village, keffi local Government Area of Nasarawa state, Nigeria. The plant was identified, authenticated and assigned a voucher number (NSUK/PLVa/004) and deposited at the Habarium, by a Taxonomist at the Department Plant Science and Biotechnology Nasarawa State University, Keffi, Nasarawa state, Nigeria. The leaves were washed under running tap water, air dried for two weeks and ground into fine powder using the electric blender. Two hundred and fifty grams (250g) of the pulverized leaves was weighed and dissolved in 800ml of absolute ethanol; the mixture was allowed to stand for a period of 48 hours with intermittent shaking. After extraction, the sample was filtered using whatmann No 1 filter paper. The filtrate was evaporated using a rotary evaporator under reduced pressure at 65°C and a semi-solid crude extract was obtained, which was subjected to for further concentration using water bath until it became solidified. The extract was divided into two places; one for phytochemical analysis and the other for oral administration, both were stored in a refrigerator at - 4^{o}C prior to use.

Phytochemical Analysis

The preliminary phytochemical screening of the ethanol extract was carried out in order to ascertain the presence of some plant secondary metabolites such as alkaloids, tannins, phenolics, saponins, flavonoids, terpenoids, steroids, glycosides and resins and those detected were further quantified. Both determinations were done by utilizing standard conventional protocols as illustrated by (Harborne, 1973; Trease and Evans, 2002; El-olemyl, 2005).

Experimental Animals

A total of sixteen (16), Wister albino rats weighing between 120 g-160 g were used for the study. The rats were housed in well ventilated cages in the animal house of the Department of Biochemistry and Molecular Biology, Nasarawa State University Keffi, Nasarawa State. The animals were allowed free access to feed (growers mash) and clean water. The animals were kept under 24 hours light/dark cycling and were equally acclimatized to the housing and feeding conditions for a period of 7 days. After acclimatization the animals were marked and separated randomly into four groups with four animals per group.

Administration of Ethanol Extract

The extract was reconstituted in distilled water into appropriate concentration by dissolving 5g of the ethanol extract in 100 ml of distilled water and the extract was partially dissolved. One group which was set as control received only feed and water. While other three groups (2, 3, 4) were administered with feed, water and 200, 300 and 400 mg/kg b.w of the ethanol extracts, respectively for a period of two weeks.

Preparation of Blood Sample in Rats

The orbital bleeding method was employed. A microhaematocrit capillary tube was inserted at the medial canthus to gain access to the orbital venous plexus of the rat since this area offers a readily accessible site for blood collection. The blood samples were collected into EDTA tubes and plain tubes and used for the estimation of the different haematological parameters and lipid profile respectively.

Preparation of Serum

Blood samples in the plain tubes were allowed to stay for 30 minutes for clotting. It was centrifuged using Denley BS400 centrifuge (Vickas Ltd, England) at 3000 rpm for 15 minutes and the serum collected and then subjected to biochemical assays.

Determination of Haematological Parameters

The packed cell volume and red blood cell count were determined using the standard method described by (Ochei and Kolhatkar, 2008). Haemoglobin, white blood cell count and platelets count were done according to the method described by Miale (1972), Dacie and Lewis (1991) and Umarani and Shashidhar (2016) respectively. Mean Cell Volume, and Mean Cell Heamoglobin Concentration were calculated using standard formula.

Estimation of lipid profile

Total cholesterol was determined according to the method of Abell *et al.*, (1952), triglyceride was also determined according to the method of Bucolo and David (1973), while LDL-Cholesterol and HDL-Cholesterol were determined by the method of Kameswara *et al.* (1999).

Statistical Analysis

Results are reported as mean $\pm$ SD and statistical analysis was performed using one way ANOVA and $P < 0.05$ is being considered as statistically significant

III. RESULTS AND DISCUSSION

RESULTS

Qualitative and quantitative phytochemical composition of V. amygdalina

The table below showed the phytochemical composition of ethanol leaf extract of *V. amygdalina.*

Table 1: Qualitative and quantitative composition of *Vernonia amygdalina*

Phytochemical	Qualitative	Quantitative (mg/100g)
Alkaloids	++	137.33±2.08
Flavonoids	++	135.33±5.69
Terpenoids	+	53.00±4.58
Steroid	-	ND
Phenolics	+	35.66±3.51
Saponins	+++	801.33±7.57
Tanins	++	256.66±30.83
Glycosides	+	38.67±2.52
Resins	++	157.60±4.04

Key: + = Present in small amount, ++ = Present in moderate amount, +++ = Present in abundant, - = Absent, ND = Not Detected. Results are expressed in Means ± SD (n = 3)

Table 2: Effect of *V. amygdalina*Leaf Extract on the Haematological Parameters of Wister Albino Rats

	PCV (%)	Hb (g/l)	RBC ($x10^{12}$/l)	WBC ($x10^{9}$/l)	PLT ($x10^{9}$/l)	MCHC (g/l)	MCV (fl)	MCH (pg)
Group 1	41.0 ± 1.8^{a}	135.80 ± 2.50^{c}	1.31 ± 0.03^{e}	1.47 ± 0.40^{g}	303.80 ± 5.10^{j}	315.80 ± 1.70^{m}	0.33 ± 0.30^{o}	102.99 ± 1.40^{s}
Group 2	43.5 ± 2.1^{a}	143.00 ± 2.90^{c}	1.73 ± 0.05^{e}	2.47 ± 0.40^{g}	146.50 ± 1.20^{k}	331.8 ± 2.80^{n}	0.26 ± 0.0^{p}	81.03 ± 1.20^{t}
Group 3	42.0 ± 1.8^{a}	138.00 ± 5.70^{c}	1.77 ± 1.00^{e}	3.64 ± 1.30^{h}	211.00 ± 1.80^{l}	330.8 ± 4.30^{n}	0.18 ± 0.0^{q}	55.67 ± 0.50^{u}
Group 4	27.2 ± 1.7^{b}	75.30 ± 15.10^{d}	0.52 ± 0.01^{f}	8.58 ± 0.50^{i}	142.50 ± 1.30^{k}	332.0 ± 2.90^{n}	0.41 ± 0.0^{r}	153.69 ± 2.80^{v}

Mean values with different letters as superscripts down the column are considered significant at $p < 0.05$

Group 1 = Control; Group 2 = 200 mg/kg b.w. of the extract; Group 3 = 300 mg/kg b.w. of of the extract; Group 4 = 400 mg/kg b.w. of of the extract

Results are expressed in Means ± SD (n = 5)

Table 3: Effect of *Vernonia amygdalina* extract on the Lipid profile of wister albino rats

	TAG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Group 1	106.89±8.20^{a}	84.21±4.82^{b}	65.99±4.43^{d}	16.71±1.35^{e}
Group 2	106.55±4.19^{a}	94.51±4.89^{c}	65.35±4.57^{d}	15.93±1.25^{e}
Group 3	99.38±4.32^{a}	97.88±6.16^{c}	67.22±3.52^{d}	15.76±5.80^{e}
Group 4	97.23±8.58^{a}	86.10±5.82^{b}	60.90±.96^{d}	14.06±3.09^{e}

Results are expressed in Means ± SD (n = 5)
Mean values with different letters as superscripts down the column are considered significant at $p < 0.05$

IV. DISCUSSION

The presence of alkaloids, tannins, phenolics, saponins, flavonoids, terpenoids, glycosides and resins were detected in the ethanol leaf extract of *V. amygdalina* which is in agreement with the findings of Cimanga, *et al.* (2004) and Eleyinmi *et al*. (2008). Saponins, tannins and resins were found to be high in the extract with saponins as the most abundant as shown in (Table 1). Saponins have been found to be potentially useful for the treatment of hypercholesterolemia which suggested that saponin might be acting by interfering with intestinal absorption of cholesterol, thus have antihypercholesterol and antidiabetic effects (Soetan and Aiyelaag, 2009 ; Ezeabara *et al.*, 2014). Saponins have also been shown to have immense significance as antihypertensive and cardiac depressant properties (Trease and Evans, 1989; Price *et al*., 1987). Plants that have tannins as their component are stringent in nature and are used for treating intestinal disorder such as diarrhea and dysentery (Fujiki, 2012; Dharmananda, 2003; Trouillas, 2003). This observation therefore, supports the use of *V. amygdalina* in herbal cure remedies. Li and Wang (2003), reviewed the biological activities of tannins and observed that tannins have anti-cancer activity and can be used in cancer prevention, thus suggesting that *V. amygdalina* has potential as a source of important bioactive molecule for the treatment and prevention of cancer. Resins were also found to be significantly higher which is in contrast to the findings of Oguwike (2013). The presence of resins suggest the partial solubility of the extract in water. Flavonoids have been reported to possess antioxidant, anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (Edeoga *et al*., 2005).The use of the leaves extract for treatment of abdominal pain and painful uterus might be as a result of

the analgesic property of alkaloid (Stray, 1998). Alkaloids have also been implicated for its detoxifying and antihypertensive properties (Trease and Evans, 1989; Zee-cheng, 1997). Furthermore, an appreciable quantity of phenol was observed giving an indication that the plant might play an important role as dietary antioxidant. Phenolic compounds prevent oxidative damage in living systems (Block, 1992); Enemali and Udedi (2018) reported the antioxidant effects of crude leaf extracts of *V. amygdalina* . The bitter taste of *V. amygdalina* was reported to be due to the presence of anti-nutritional factors such as alkaloids, saponins, tannins and glycosides (Arhoghro *et al*., 2009). Glycosides are important class of naturally occurring compounds whose actions help in the treatment of congestive heart failure (Yukari *et al.,* 1995).Terpenoids was found to be significantly ($p < 0.05$) low, the antimicrobial activity of the extracts may be attributed to the presence of terpenoids (Böhme *et al.,* 2014) and other secondary metabolites. Terpenoids are known to have this activity due to the possible effect on the non - mevalonate pathway. Steroid was found to be absent, this is in contrary to the report of Udochukwu *et al*., (2015) and Usunobun and Okolie (2016). These may be probably due to differences in location, age of the plant, period of harvest or mode of extraction.

Following administration of the extract, there was a significant reduction ($p < 0.05$) in the blood levels of erythrocyte parameter which was observed at the highest dosage (400mg/kg b.w) especially for RBC, Hb and PCV counts, this finding revealed that *V. amygdalina* may have a possible potential to inhibit erythropoietin release from the kidneys, which is the humoral regulator of RBC production and also affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and haemoglobin are very important in transferring respiratory gases (Polenakovic and Sikole, 1996; Oyedeji *et al.,* 2013). The reduction might be due to the presence of saponins, which has been reported to reduce haematological parameters probably due to breakdown of blood cells or suppression of blood synthesis (Schneider *et al.,* 2003). But at 200 and 300mg/kg b.w there was a non-significant change ($p>0.05$) in the RBC, Hb and PCV values This is an indication that there may be no destruction of RBCs and no pronounced change in the rate of production of RBCs (erythropoiesis) therefore, suggesting the non - toxic nature of the plant extracts to RBCs at this dosage. WBC levels increased significantly ($p < 0.05$) at 300mg/kg and 400mg/kg extract concentrations which show that the extract has an immune boosting property. The effect of the extracts on the total WBC count could be due to the presence of saponins. This compound (saponins glycosides) has an anti-inflammatory property and so has vital effect on inflammatory processes of some pathological states such as bacterial infection, malaria and liver diseases (Ugochukwu and Babady, 2002). This finding is in agreement with the work of Sweeney *et al*, (2005) which suggested that *V. amygdalina* extract may strengthen the immune system through many cytokines regulation. Also, the extract was shown to significantly ($p < 0.05$) decrease platelet cells in all the treatment groups when compared to the control, the significant change in the platelet count caused by the extract could be an indication that it does not have the potential to stimulate thrombopoietin production (Li *et al.,* 1999) with the haemostatic capability of the blood maintaining the *status quo* since platelets mediate in the blood–clotting

mechanism. MCHC concentrations were however observed to increase significantly ($p < 0.05$) in all the test groups when compared to the control, indicating relative high concentration of hemoglobin thereby suggesting an elevation in disease such as hereditary spherocytosis, sickle cell disease and homozygous hemoglobin C disease. Also MCV and MCH values which were observed to be significantly ($p < 0.05$) lower in groups 2 and 3 indicating a microcytic anemia (suggesting an interference with iron uptake into hemoglobin) but increased significantly ($p < 0.05$) in group 4 when compared to the control, which is an indicative of macrocytic anemia suggesting deficiency in vitamin B_{12} and folate.

The results in (Table 3) above revealed that the ethanol leaf extract of *V. amygdalina* has no remarkable effect on the lipid profile of the rats which is consistence with previous findings of Erasto *et al*. (2007) and Ajuru *et al*. (2013) and Enemali and Udedi (2018). However this report is in contrast to the work of Kadiri (2017) who state that irrespective of the dose, *V. amygdalina* is able to improve the lipid profile in cyanide exposed rats. Also Arit *et al*. (2007) reported that bitter leaf extract greatly improved the lipid profile of albino rats. The observed non-significant ($p > 0.05$) decrease in serum level of TAG, HDL-C and LDL-C may be attributed to the effect of saponins. Saponins are known antinutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption and/or by binding with bile acids, causing a reduction in the entero-hepatic circulation of bile acids and increase its fecal excretion (James *et al*., 2010; Rotimi *et al*., 2011). Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol (Rotimi *et al*., 2011). Hence, saponins have been reported to have hypocholesterolic effect (James *et al*., 2010). Presence of saponins has been reported in the phytochemical screening of the extract among other polyphenolic compounds, these may explain the antihyperlipidemic effect observed in this study. Previous studies showed that phytochemicals such as alkaloids, saponins, flavonoids and polyphenols are known to reduce serum lipid level in animals (Ezekweand Obioha, 2001). However, TC increased significantly ($p < 0.05$) in groups 2 and 3 when compared to control but, the increase in group 4 was not statistically significant ($p > 0.05$) when compared to the control this may be attributed to the presence of other polyunsaturated fat or fatty acids.

V. CONCLUSION

From the results of the study, it can therefore, be concluded that the bitter leaf extract is rich in bioactive compounds, but posed negative effect on the haematological parameters at 400mg/kg b.w with no remarkable effect on the lipid profile at the administered doses within the period of the research.

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