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Modulatory Effects of *Vernonia amygdalina* Leaf Extract on Hematological Parameters in Rats Exposed to 1,2-Dimethylhydrazine-Induced Toxicity

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ABSTRACT

Background and Objective: *Vernonia amygdalina*, commonly known as bitter leaf, is a shrubby plant native to Tropical Africa that has gained global attention from researchers and scientists due to its potential health benefits and medicinal applications. This study was conducted to investigate the modulatory effects of *Vernonia amygdalina* leaf extract on hematological indices in rats exposed to 1,2-dimethylhydrazine-induced toxicity. **Materials and Methods:** Forty male Wistar albino rats within the weight range of 150-200 g were assigned to eight groups (5 rats per group): Control, DMH, silymarin, *VA* only, pretreatment (200 mg/kg bwt), pretreatment (400 mg/kg bwt), post-treatment (200 mg/kg bwt), and post-treatment (400 mg/kg bwt) groups, respectively. Except for the control and *VA* only groups, the rats were exposed to DMH before or after treatment with *VA* via the intraperitoneal route at a single dose of 40 mg/kg bwt. At the closing stages of the experiment, blood samples were collected in EDTA bottles for hematological assays. Treatment lasted for 21 days. Data were expressed as Mean±Standard error mean and considered significant at $p<0.05$. All statistical analyses were done using the SPSS software (version 20). **Results:** Treatment with *Vernonia amygdalina* significantly improved final body weight compared to the DMH group ($p<0.05$). Hematological analysis showed significant reductions in RBC, HB, LYM, MCV, MCHC, MID, and PLT in the DMH group. Pre- and post-treatment with VA moderately restored these parameters, though changes were mostly insignificant. WBC, PCV, MPV, and related indices increased in the DMH group versus control and some treated groups. **Conclusion:** The findings from this study indicate that *Vernonia amygdalina* leaf extract is effective against blood toxicity caused by DMH, and it could serve as a benchmark for further studies.

KEYWORDS

Toxicity, intraperitoneal, *Vernonia amygdalina*, ethanol extract, medicinal plant, single dose

INTRODUCTION

Hematotoxicology occurs as a result of the adverse effects of chemicals on blood and blood-forming tissues, which alter the pivotal roles played by blood cells, making them highly susceptible to intoxication[1]. It involves the degree of damage caused by these chemicals to the exposed tissues,

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ultimately compromising the overall health and function of the organism[2]. Tissues are exceptionally vulnerable to toxic insults due to the crucial functions performed by blood cells. However, some important processes such as nutrient delivery (e.g., iron), toxin and metabolite removal (e.g., urea), or the production of vital growth factors like erythropoietin and Granulocyte Colony-Stimulating Factor (G-CSF), can exert detrimental effects on blood cells, eventually compromising their function and overall health[3].

A 1,2-Dimethylhydrazine (DMH) is a powerful chemical toxicant that reliably induces organ tumors in experimental animal models. As the most extensively utilized model of chemically induced toxicity, DMH exposure culminates in carcinogenesis, making it a valuable tool for investigating the complex mechanisms underlying cancer development[4]. A 1,2-Dimethyl hydrazine shares many resemblances to human colorectal toxicity, including resemblance in the response to some promotional and preventive agents[5]. Models used in DMH toxicity studies are developed for exploring the medicinal properties of plant-derived components, because they offer identical site and pathological changes, which can also be found in humans[6].

Vernonia amygdalina, a member of the daisy family, is a small shrub that grows in tropical Africa and possesses much economic significance[7]. Bitter leaf, as it is popularly known, is a medicinal plant widely used in traditional medicine across various parts of Nigeria. The leaves of this plant, which have numerous bioactive compounds, offer a wide range of potential health benefits[8]. *Vernonia amygdalina* is rich in biologically active compounds such as antioxidants and polyphenols, which helps to reduce oxidative stress resulting from the accumulation of free radicals, thus leading to the prevention of diseases[9]. The study aims to investigate the modulatory effects of *Vernonia amygdalina* leaf extract on hematological indices in rats exposed to 1,2 dimethylhydrazine-induced toxicity.

MATERIALS AND METHODS

Study area: This study commenced on November 10th, 2024, and ended on December 12th, 2024 (i.e., a period of 4 weeks, 2 days). The laboratory experiment was carried out at the laboratory section of the Department of Biochemistry, University of Benin, Benin, Edo, Nigeria.

Experimental animals: Forty Wistar albino rats were purchased from the animal house, Department of Biochemistry, University of Benin, Benin. The sexes and weights of the rats were determined, and they were males with body weights ranging from 150-200 g. The rats were accommodated in clean, disinfected cages under standard laboratory conditions, with access to feeds (pelletized growers mash) and water *ad libitum*. They were acclimatized for 2 weeks before the experiment began.

Plant collection, authentication, and extract preparation: Fresh mature leaves of *Vernonia amygdalina* obtained from a vegetable farm in Benin, Edo, were identified and authenticated in the Herbarium of the Department of Plant Physiology and Biotechnology, University of Benin, Benin, Edo, Nigeria. The leaves were separated from the stalk, washed and air-dried at room temperature, pulverized, crushed into fine powder, and weighed. Ethanol extract of the leaves was prepared by soaking 400 g of the powdered plant leaves in 1 L of absolute ethanol at room temperature for 72 hrs. The extract was thereafter filtered first through Whatman filter paper No. 42 (125 mm) and concentrated with a rotatory evaporator at 40°C. The concentrated extract was subsequently freeze-dried via lyophilization.

Experimental design/protocol: The rats were randomly assigned to 8 groups of 5 rats each as follows: Control, DMH, silymarin, *VA* only, pretreatment (200 mg/kg bwt), pretreatment (400 mg/kg bwt), post-treatment (200 mg/kg bwt), and post-treatment (400 mg/kg bwt) groups, respectively. Except for the control and *VA* only groups, the rats were exposed to DMH before or after treatment with *VA* via the intraperitoneal route at a single dose of 40 mg/kg bwt[10]. Rats in the silymarin group were treated with the standard hepato-/cardio-protective drug, silymarin (100 mg/kg bwt). Treatment lasted 21 days (3 weeks).

Ethical statement: The experimental protocol was approved by the Faculty of Life Science Ethical Committee of the University of Benin, Benin, Edo, Nigeria.

Sample collection: At the end of the treatment period, blood samples were collected via cardiac puncture under mild ketamine anesthesia into plain and heparin/EDTA containers. The blood was centrifuged at 3500 rpm for 15 min to obtain plasma.

Biochemical assays: The assay on hematological analysis was conducted[11]. Horiba ABX 80 hematology analyzer was used for the measurement of hematological parameters following the manufacturer's instructions; RBC, WBC, HB, LYM, MPV, PCV, MCV, MCHC, PLT, GRAN, MCH, PCT, PDW, P-LCR, RDW-CV, and RDW-SD.

Data analysis: The results obtained from this study were evaluated using the One-way Analysis of Variance (ANOVA). Data was expressed as Mean±Standard error mean (n = 5). For each parameter, values having different superscripts between groups differ significantly ($p<0.05$). A *post-hoc* comparison test was carried out using the Tukey's HSD test to evaluate pair-wise differences among group means. All statistical analyses were done using the SPSS software (version 20).

RESULTS

Results obtained for the body weight of rats, as shown in Table 1, indicated that the DMH group recorded significant reductions ($p<0.05$) in body weight when compared with the control and the other groups. Consequently, there was a significant increase ($p<0.05$) in the body weight of rats in the treatment groups when compared with the DMH group. Also, the silymarin group recorded a significant increase ($p<0.05$) in final body weight when compared with the other groups.

As shown in Table 2, there was a percentage decrease ($p<0.05$) in the body weight change of rats in the DMH group when compared with control and the other groups. Furthermore, groups treated with ethanol extract of *V. amygdalina* leaves, before and after DMH administration recorded percentage increase ($p<0.05$) in the body weight change relative to the DMH group.

Table 1: Initial and final body weights of rats in different treatment groups

Group	Initial weight (g)	Final weight (g)
Control	170.87±8.12^{a}	200.38±14.48^{a}
DMH	195.03±12.01^{b}	178.95±11.17^{b}
Silymarin	182.39±5.20^{c}	212.42±13.56^{ac}
VA only	180.44±3.42^{c}	216.08±11.94^{c}
Pretreatment (200 mg/kg bwt)	170.36±35.09^{a}	188.86±41.39^{d}
Pretreatment (400 mg/kg bwt)	168.15±34.62^{d}	187.66±16.35d^{e}
Post-treatment (200 mg/kg bwt)	177.66±6.87^{ae}	195.44±7.48^{f}
Post-treatment (400 mg/kg bwt)	166.50±6.47^{df}	12.37^{ag}

Values are stated as Mean±SEM (n = 5) and For each parameter, values having different superscripts between groups differ significantly ($p<0.05$)

Table 2: Change and percentage change in body weight of rats following DMH and *Vernonia amygdalina* treatments

Groups	Weight change (g)	Weight change (g) (%)
Control	29.51±6.36	17.27±0.78
DMH	16.08±0.84	8.24±0.06
Silymarin	30.03±8.36	16.46±1.60
VA only	35.64±8.52	19.75±2.49
Pretreatment (200 mg/kg bwt)	18.50±6.30	13.18±0.17
Pretreatment (400 mg/kg bwt)	41.51±18.27	30.04±0.53
Post-treatment (200 mg/kg bwt)	17.78±0.61	10.00±0.88
Post-treatment (400 mg/kg bwt)	38.02±5.90	0.91

Values are stated as Mean±SEM (n = 5) and For each parameter, values having different superscripts between groups differ significantly ($p<0.05$)

Table 3: Red and white blood cell counts and hemoglobin concentration in rats treated with DMH and *Vernonia amygdalina*

Group	Red blood cells ($\times 10^6$ uL^{-1})	White blood cells ($\times 10^3$ uL^{-1})	Haemoglobin (g dL^{-1})
Control	7.61±0.14^a	4.40±0.80^a	15.15±0.55^a
DMH	7.50±0.22^a	4.80±0.20^b	15.10±0.40^a
Silymarin	7.47±0.03^a	2.75±0.55^c	15.60±0.50^b
A only	7.59±0.28^a	3.85±0.25^d	15.90±0.70^b
Pretreatment (200 mg/kg bwt)	7.31±0.73^a	4.10±1.10^a	16.45±1.35^c
Pretreatment (400 mg/kg bwt)	7.99±0.33^b	3.80±1.50^d	16.65±0.35^c
Post-treatment (200 mg/kg bwt)	7.31±0.09^b	3.50±0.40^d	14.45±0.75^d
Post-treatment (400 mg/kg bwt)	8.27±0.12^c	4.70±1.80^b	0.15^e

Values are stated as Mean SEM (n = 5) and For each parameter, values having different superscripts between groups differ significantly ($p<0.05$)

Table 4: Lymphocyte count, mean platelet volume, and packed cell volume in treated and control rats

Group	Lymphocytes (%)	Mean Platelet Volume (FL)	Packed Cell Volume (%)
Control	92.50±2.20^a	7.80±0.10^a	48.60±2.70^a
DMH	91.50±2.30^b	8.15±0.35^b	49.95±1.25^b
Silymarin	92.45±1.55^a	7.60±0.50^c	47.75±1.35^c
VA only	91.25±3.95^b	7.60±0.00^c	49.35±0.25^b
Pretreatment (200 mg/kg bwt)	95.35±0.15^c	7.90±0.10^d	47.75±3.65^c
Pretreatment (400 mg/kg bwt)	91.80±1.80^b	7.35±0.05^e	50.00±1.40^d
Post-treatment (200 mg/kg bwt)	94.30±0.90^d	7.65±0.15^c	47.90±0.90^c
Post-treatment (400 mg/kg bwt)	94.00±1.80^d	7.85±0.15^a	53.90±3.30^{de}

Values are stated as Mean SEM (n = 5) and For each parameter, values having different superscripts between groups differ significantly ($p<0.05$)

Table 5: Mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration across experimental groups

Group	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
Control	63.86±0.19^a	19.90±0.39^a	31.17±0.20^a
DMH	66.60±0.56^b	20.13±0.02^b	30.23±0.32^b
Silymarin	63.92±0.27^a	20.88±1.66^b	32.65±0.37^c
VA only	65.01±0.08^c	20.95±0.25^b	32.21±0.28^c
Pretreatment (200 mg/kg bwt)	65.32±0.50^c	22.50±0.18^c	34.45±0.37^d
Pretreatment (400 mg/kg bwt)	62.57±0.42^c	20.83±0.11^b	33.30±0.25^e
Post-treatment (200 mg/kg bwt)	65.52±1.00^c	19.76±0.83^a	30.16±0.83^b
Post-treatment (400 mg/kg bwt)	65.17±0.75^d	21.22±0.13^d	32.56±0.05^c

Values are stated as Mean SEM (n = 5) and For each parameter, values having different superscripts between groups differ significantly ($p<0.05$)

Table 6: Platelet count, MID percentage, and granulocyte levels in rats exposed to DMH and *Vernonia amygdalina*

Group	PLATELET ($\times 10^9$/L)	MID (%)	GRAN (%)
Control	336.51±3.30^a	19.52±2.10^a	8.13±0.17^a
DH	126.26±2.77^b	15.33±1.20^b	15.28± 3.32^b
Silymarin	372.30±4.31^a	20.13±2.42^c	9.01±2.11^c
VA only	306.15±3.11^a	17.10±1.84^d	9.32±2.21^c
Pretreatment (200 mg/kg bwt)	387.22±4.00^a	19.49±2.03^a	14.11±0.42^d
Pretreatment (400 mg/kg bwt)	321.42±3.04^a	18.54±0.19^e	9.40±2.10^a
Post-treatment (200 mg/kg bwt)	352.39±3.15^a	18.22±0.45^e	9.07±3.15^a
Post-treatment (400 mg/kg bwt)	390.88±3.63^a	16.15±2.45^f	10.22±2.48^e

Values are stated as Mean SEM (n = 5) and For each parameter, values having different superscripts between groups differ significantly ($p<0.05$)

The results obtained for the hematology studies as shown in Table 3-8 revealed a decrease ($p<0.05$) in the mean values of RBC, HB, LYM, MCH, MCHC, MID, and PLT in the DMH group (40 mg/kg body wt.) when compared with control, the pre-treatment (200 mg/kg), and the post-treatment groups (400 mg/kg), respectively, but the differences between the groups were insignificant. Consequently, the Silymarin (100 mg/kg) and *VA* (300 mg/kg) groups recorded insignificant changes in mean values when compared with the control and DMH groups, respectively. However, there was an insignificant increase in the

Table 7: Plateletcrit, platelet-large cell ratio, and platelet distribution width in experimental rats

Groups	PCT	P-LCR (%)	PDW (%)
Control	0.52±0.13[a]	15.12±4.01[a]	8.37±0.62[a]
DMH	0.86±0.02[b]	39.30±6.17[b]	13.50±0.10[b]
Silymarin	0.70±0.10[c]	22.74±5.26[c]	10.21±0.04[c]
VA only	0.57±0.13[a]	22.93±6.03[c]	10.33±0.55[c]
Pretreatment (200 mg/kg bwt)	0.42±0.03[c]	27.43±6.69[c]	10.50±0.08[c]
Pretreatment (400 mg/kg bwt)	0.70±0.12[d]	24.50±2.36[c]	12.35±0.65[d]
Post-treatment (200 mg/kg bwt)	0.63±0.20[e]	27.37±6.06[c]	10.70±0.80[c]
Post-treatment (400 mg/kg bwt)	0.70±0.90[d]	20.22±5.26[c]	9.39±1.21[e]

Values are stated as Mean SEM (n = 5) and For each parameter, values having different superscripts between groups differ significantly (p<0.05)

Table 8: Red cell distribution width-coefficient of variation and standard deviation in rats from different treatment groups

Group	RDW-CV (%)	RDW-SD (fL)
Control	12.47±0.35[a]	24.61±0.22[a]
DMH	21.12±1.22[b]	32.70±0.40[b]
Silymarin	18.47±1.14[c]	30.50±1.11[c]
VA only	20.46±0.38[d]	26.53±0.22[a]
Pretreatment (200 mg/kg bwt)	20.34±1.17[d]	30.07±1.72[c]
Pretreatment (400 mg/kg bwt)	19.22±1.20[e]	30.12±1.16[c]
Post-treatment (200 mg/kg bwt)	19.58±1.23[e]	30.90±1.83[c]
Post-treatment (400 mg/kg bwt)	14.10±0.29[f]	29.20±1.31[a]

Values are stated as Mean SEM (n = 5) and For each parameter, values having different superscripts between groups differ significantly (p<0.05)

mean values of the RBCs, HB, and LYM in the pre- and post-treatment groups relative to the DMH group. Also, there was an increase in the mean values of WBCs, PCV, MPV, MCV, PDW, PCT, P-LCR, GRAN, RDW-CV, and RDW-SD in the DMH group when compared with the control and one of the pretreatment groups.

DISCUSSION

In this current research, the rats were exposed to 1,2 dimethylhydrazine (DMH before and after *Vernonia amygdalina* administration. Results obtained for the body weight of rats, as shown in Table 1, indicated that the DMH group recorded significant reductions in the final body weight when compared with the control and the other groups (p<0.05). When rats are exposed to toxicants, they typically experience a decrease in body weight compared to control rats, meaning the exposure to DMH can lead to weight loss in the animals; this is often used as an indicator of toxicity and is frequently studied in research investigating potential preventive treatments for diseases[12]. The percentage increases in body weight of rats treated with ethanol extract of *V. amygdalina* leaves were significantly increased relative to the DMH group as shown in Table 2 (p<0.05). This implies that the extract possesses beneficial properties for weight management. This agrees with a similar previous study by Umeaku *et al.*[13].

Hematological indices which measure red blood cell count, white blood cell count, platelet count, and hemoglobin levels, are crucial for assessing the overall health of the body and detecting potential diseases. These indices can be influenced by various factors, including diet, exposure to toxins, and disease conditions[14]. The results obtained for the hematology studies, as shown in Table 3-6 revealed a decrease in the mean values of RBC, HB, LYM, MCHC, MID, and PLT in the DMH group (40 mg/kg bwt.) when compared with control, the pre-treatment (200 mg/kg bwt.), and the post-treatment groups (400 mg/kg bwt.), respectively, but the differences between the groups were insignificant (p<0.05). Consequently, the Silymarin (100 mg/kg) and *VA* (300 mg/kg) groups recorded insignificant changes in mean values when compared with the control and DMH groups, respectively. These reductions in the DMH group reveals the inability of the immune system to respond effectively to infections or excessive bleeding, which leads to anemia[15]. However, there was an insignificant increase in the mean values of the RBCs, HB, and LYM in the pre and post-treatment groups relative to the DMH group, as shown in Table 3-4, respectively (p<0.05). The findings from this result shows that *V. amygdalina* can elicit the release of erythropoietin 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 hemoglobin (Hb) are very essential in transferring respiratory gases[16,17]. The increase in the values of the lymphocytes is associated with the ability of the animals to perform well under very stressful conditions. These suggest that the phytochemical compounds present in the extracts elicited stress responses, and it has a vital effect on inflammatory processes of some pathological states such as bacterial infection, malaria, and liver diseases[18]. The significant increases in platelet count observed for treatment groups, as shown in Table 6 ($p<0.05$), is an indication of the probable ability of the extract to activate the actions of platelet activating factor (PAF) especially when administered at a longer duration, hence improving the blood clotting potentials[19]. Consequently, there was an increase in the mean values of WBCs, PCV, MPV, MCV, PDW, PCT, P-LCR, GRAN, RDW-CV, and RDW-SD in the DMH group when compared with control and one of the pretreatment groups, as shown in Table 3-8 ($p<0.05$). The increase in these parameters occurred as a result of provoked responses by the immune system when certain illnesses or conditions are encountered in the body system, leading to an increase or decrease in the quantity of the parameters when in circulation[20]. These values were slightly decreased especially in the silymarin, pre- and post-treatment groups ($p<0.05$). Also, the insignificant decrease in the WBCs may be due to the administration of *V. amygdalina,* which has been shown to have immune modulating properties ($p<0.05$)[21].

CONCLUSION

The results of this study have shown that *Vernonia amygdalina* is able to improve the hematopoietic system of rats and reduce oxidative stress associated with blood toxicity in rats. This suggests that the phytochemical compounds present in the extracts elicited stress responses from blood parameters, which have a vital effect on inflammatory processes of some pathological states such as bacterial infection, malaria, and liver diseases. Thus, further research on *Vernonia amygdalina* may reveal its potential as a source of phyto-medicine, which can be presented as a recommendation for clinical trials.

SIGNIFICANCE STATEMENT

This study investigated the protective effects of *Vernonia amygdalina* (bitter leaf), a plant commonly used in traditional medicine, against the toxic effects of 1,2-dimethylhydrazine, a chemical that can cause damage to the blood and other organs. The results showed that *Vernonia amygdalina* leaf extract significantly improved blood cell counts and reduced the levels of toxic chemicals in the body. These findings suggest that *Vernonia amygdalina* may be a valuable natural remedy for preventing or treating blood disorders caused by exposure to toxic chemicals. This discovery has important implications for public health, particularly in communities where exposure to toxic chemicals is a significant risk. By exploring the potential health benefits of *Vernonia amygdalina*, we can develop new, sustainable approaches to preventing and treating diseases, ultimately improving the health and well-being of individuals and communities.

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