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# Effect of *Vernonia amygdalina* on Haematological Indices and Kidney Function in Rats Exposed to Stress

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**ABSTRACT**

This study investigated the effect of V. amygdalina on the hematological parameters and kidney function in Wistar rats exposed to stress. 25 Wistar rats were randomly assigned to five groups. NCG (control) was given water only, $T_1S_0$ was given V. amygdalina extract but not stressed, $T_2S_{24}$, $T_3S_{36}$, and $T_4S_{48}$ were given same concentration of V. amygdalina extract (100mg/kgBW) and stressed for 24hours, 36 hours and 48hours respectively. Hematological test was carried out by Mindray Haematology Analyzer BC-2800 and creatinine/urea tests were performed using Randox method. The result reveals increased WBC count in all treatment groups, which was significant only ($P<0.05$) in treatment group $T_4S_{48}$=21.28x$10^3$/$mm^3$. The Hb concentration significantly increased in $T_1S_0$=14.86 x$10^3$/$mm^3$ and $T_2S_{24}$=14.88 x$10^3$/$mm^3$ respectively. There was significant ($P<0.05$) increase in serum creatinine ($T_1F_0$=0.196*, $T_2F_{24}$=0.191* and $T_3F_{36}$=0.175*) and urea concentration ($T_1S_0$=52.1* and $T_2S_{24}$=48.6*) when compared with their control. The result further revealed that increased duration of fasting stress progressively decreased serum creatinine ($T_2F_{24}$=0.191, $T_3F_{36}$=0.175 and $T_4F_{48}$=0.164) and urea ($T_2S_{24}$=48.6, $T_3F_{36}$=36.4 and $T_4S_{48}$= 26.0) concentration. These findings indicates that the boiled bitter leaf extract is able to improve hematological parameters but may compromise kidney function and that increased duration of fasting may have some reno-protective effects.



## 1. INTRODUCTION

The usefulness of plant to man is not only as a source of raw materials for industries, but also as source of food and medication. From time immemorial, plants have provided man with diverse means of healing. They have been known to possess abundant phytochemicals and pharmacologically active properties which include; anthraquinones, flavonoids, saponins, polyphenols, tannins and alkaloids [1]. In medical or

biological context, stress is a physical, mental or emotional factor that causes bodily or mental tension. Stress is how the brain responds to any demand such as traumatic event, significant life changeor exertion at work or school [2]. There are different types of stress ranging from routine to sudden negative change stress to traumatic stress, and these are accompanied with physical and mental health risks [2]. However, not all stress are bad as some could be lifesaving preparing the body to initiate the fight or flight response, a complex reaction of neurologic and endocrinologic systems. Caloric restriction popularly called fasting is an important stress that has been associated with increased levels of cortisol and epinephrine [3]. The pharmacological study of Vernonia amygdalina (V. amygdalina) have been reported by [4] to demonstrate antihelmintic and antimalarial properties, antitumorigenic properties by [5], analgesic and antipyretic activities by [6], hypoglycemic and hypolipidaemic effect in experimental animals. The biologically active phytoconstituents from V. amygdalina are alkaloids, flavonoids, terpenes, saponins, coumarins, xanthones, phenolic acids, lignans, steroids, anthraquinones as reported by [7], edotides [8], and sesquiterpene lactone. The hypoglycemic, anti-diabetic and anti- cholesterol properties of V. amygdalina is particular importance as many locals utilize the leaves in management of diabetes. Despite the vast traditional use of V. amygdalina, it is still thought-about among the beneath exploited crops of economic significance. Considering the employment of the ironweed amygdalina leaves by the overall public, the study thus seeks to verify whether or not the leaves of the plant once employed in the management of diabetes will reverse the venomous effects of the malady on the excretory organ for public awareness.

Hence, the aim of the study is to investigate the effects of V. amygdalina on hematological indices and kidney function in stress induced male wistar rats.

## 2. METHODS

### *2.1 Preparation of bitter leaf extract*

Fresh bitter leaves (V. amygdalina) were gotten from a local farm, identified and authenticated by a Taxonomist. The leaves were washed and air dried before pulverized into fine powder with Corona Manual Grinder. 250g of the powdered sample was soaked with 2L of boiled water and stirred using the stir rod and left for 24hrs. After 24hrs, it was filtered and the filtrate was evaporated at 78°c using a water bath (Techmel and Techmel, 420 USA). The extracts obtained were then stored in sample containers and refrigerated for further analytical procedures.

### *2.2 Study Design and Animals*

The experimental study utilized 25 male Wistar rats (Rattus norvegicus) weighing between 150 to 200g procured from the animal house of the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria were used for this experiment. The rats were randomly assigned into five groups (n=5/each) and were maintained in standard cages housed in a well-ventilated animal house with a 12:12 hour's light/dark cycle exposure at room temperature. All efforts were used to reduce the number of rats used in the experiments. Normal control group (NCG) received 100mg/kgBW normal saline and normal rat chow daily, Positive control group ($T_1S_0$) received 100mg/kgBW of aqueous bitter leaf extract (ABLE) and normal rat chow daily without being exposed to stress, Treatment fasting group 1 ($T_2S_{24}$) exposed to fasting stress for 24hrs received 100mg/kgBW of ABLE and normal rat chow daily, Treatment fasting group 2 ($T_3S_{36}$) exposed to 36hrs fasting stress received 100mg/kgBW of ABLE and rat chow daily and Treatment fasting group 3 ($T_4S_{48}$) exposed to 48hrs fasting stress received 100mg/kgBW of ABLE and rat chow daily.

Following the duration of the experimental period, the rats was anaesthetized with diethyl ether and whole blood was collected by cardiac puncture into EDTA containing tubes and serum separator tubes (SST®).

### *2.3 Ethical Approval*

The study was approved by the Faculty of Biosciences Ethics Committee, Nnamdi Azikiwe University Awka (037/08/2019, August 2019) and its procedures adheres with the experimental guidelines of the U.S. National Institute of Health (NIH) and Institutional Animal Ethics Committee (IAEC) on the care and use of laboratory animals.

### *2.4 Determination of hematological parameters*

Methodology for the hematological parameters (WBC, Hb) was carried out using Mindray Hematology Analyzer BC-2800.

The UPS was turned on then followed by the power button on the Analyzer machine, the self-check, auto check and background check was automatically performed. Once the Analyzer is at the ready state, the blood samples being collected in an EDTA container was mixed properly by inversion not by vigorous shaking. The cover of the container was opened and set to the sample probe and the start switch of the Analyzer was pressed and the container still at the sample probe until it has sucked enough sample and moved in, then the tube was removed. It began to automatically analyze and displayed the results thereafter. The print button was pressed and the results printed.

### *2.5 Methodology for Creatinine test*

0.5ml each of RIa (Piric acid) and RIb (Sodium hydroxide) was added into 25 different test tubes and the standard was added in a different test tube. 100ul of the blood serum was added in each of the test tubes and shaked vigorously. After 30seconds, the Absorbance (A1) was taken for both the samples and standard (twice), before the samples were diluted with 0.5ml of distilled water and after 2 seconds, another Absorbance (A2) was taken using the UV/visible spectrophotometer.

The A2 serves as sample, the first absorbance value serves as standard, the concentration standard widely used is 0.158.

Creatine = sample× conc. Standard /standard.

### *2.6 Methodology for Urea Test*

Using Randox method

100ul serum was added into the test tube and the 0.5ml of the standard into a test tube and labelled, then for Blank, 0.5ml of distilled water was added. Add 10ul Rib into each of the test tubes. After 10mins, 1.5ml of R2 was added into all the sample test tubes including the standard and blank. Then add 10ul of R3 into all the test tubes. The contents were shaked vigorously which gave a light blue coloration. After 15mins, the absorbance was read.

Urea= sample × conc. Standard/ standard.

### *2.7 Statistical analysis*

Data collected were analyzed as descriptive statistics of means and standard deviation and inferential statistics of ANOVA (one- way ANOVA) and Turkey's HSD to determine pairwise comparison in ANOVA data using MaxStat (version 3.60) statistical software. A p-value of $\leq 0.05$ was thought-about significant.

## 3. RESULTS

25 wistar rats divided into traditional management cluster (NCG) given solely placebo (normal saline), positive management cluster ($T_1S_0$) given 100mg/kgBW of ABLE while not stress induction and treatment teams ($T_2S_{24}$, $T_3S_{36}$, $T_4S_{48}$) given 100mg/kgBW of ABLE and stressed for 24, 36 and 48 hours respectively. Total WBC, hemoglobin concentration, creatinine and urea concentration were evaluated.

### *3.1 Effect of ABLE administration on WBC*

Table 1 reveals the effect of aqueous extract of bitter leaf (ABLE) on white blood cells (WBCs) ($10^3$ /$mm^3$) of the animals. There was marginal increase in the mean WBC count in all the treatment groups ($T_1S_0$=17.88x$10^3$/$mm^3$, $T_2S_{24}$=17.80x$10^3$/$mm^3$, $T_3S_{36}$=17.28x$10^3$/$mm^3$ and $T_4S_{48}$=21.28x$10^3$/$mm^3$), however, the WBC count was only statistically significant (P<0.05) in treatment group $T_4S_{48}$=21.28x$10^3$/$mm^3$* when compared with the control NCG=16.78x$10^3$/$mm^3$.

**Table 1:** Shows the effect of aqueous extract of Vernonia amygdalina on White blood cell count ($10^3$/$mm^3$)

| **Specimens** | **Groups ($10^3$/$mm^3$)** | | | | |
|---|---|---|---|---|---|
| | **NCG** | **$T_1S_0$** | **$T_2S_{24}$** | **$T_3S_{36}$** | **$T_4S_{48}$** |
| **A** | 14.2 | 18.5 | 19.1 | 17.3 | 19.8 |
| **B** | 17.5 | 16.8 | 16.3 | 18.2 | 22.5 |
| **C** | 19.3 | 18.6 | 17.5 | 16.1 | 19.3 |
| **D** | 15.1 | 18.2 | 18.4 | 16.7 | 21.6 |
| **E** | 17.8 | 17.3 | 17.7 | 18.1 | 23.2 |
| Mean±SD | **16.78±2.08** | **17.88±0.79** | **17.80±1.05** | **17.28±0.90** | **21.28±1.69*** |
| Common Mean | | | **18.20** | | |
| p-value | | **0.00047** | | | |
| f. ratio value | | **8.098** | | | |

*Statistically significant increase at p<0.05 with respect to control
# Statistically significant decrease at p<0.05 with respect to control

### *3.2 Effect of ABLE administration on Hb concentration*

Table 2 shows the effect of aqueous extract of bitter leaf (ABLE) on hemoglobin concentration (Hb Concentration) (g/dl) of the wistar rats. The statistics reveals marginal increase in the mean Hb concentration in all the treatment groups ($T_1S_0$=14.86, $T_2S_{24}$=14.88, $T_3S_{36}$=13.54 and $T_4S_{48}$=12.58), however, this result was statistically significant in groups $T_1S_0$=14.86*, and $T_2S_{24}$=14.88* respectively when compared with the control NCG=12.26.

**Table 2:** Shows the effect of aqueous extract of Vernonia amygdalina on hemoglobin count (g/dl)

| **Specimens** | **Groups** | | | | |
|---|---|---|---|---|---|
| | **NCG** | **$T_1S_0$** | **$T_2S_{24}$** | **$T_3S_{36}$** | **$T_4S_{48}$** |
| **A** | 11.9 | 14.5 | 15.1 | 12.5 | 12.7 |
| **B** | 12.6 | 14.9 | 14.6 | 12.9 | 13.2 |
| **C** | 13.1 | 15.4 | 14.8 | 14.5 | 11.1 |
| **D** | 12.1 | 14.3 | 15.7 | 13.8 | 12.3 |
| **E** | 11.6 | 15.2 | 14.2 | 14.0 | 13.6 |
| Mean±SD | **12.26±0.59** | **14.86±0.46*** | **14.88±0.56*** | **13.54±0.82** | **12.58±0.96** |
| Common Mean | | | **13.6** | | |
| p-value | | **0.00001** | | | |
| f. ratio value | | **15.261** | | | |

*Statistically significant increase at p<0.05 with respect to control

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# Statistically significant decrease at p<0.05 with respect to control

### *3.3 Effect of ABLE administration on Creatinine concentration*

Table 3 shows the effect of aqueous extract of bitter leaf (ABLE) on Creatinine concentration (g/dl) of the wistar rats. The results revealed that the mean creatinine concentration were significantly (P<0.05) increased in treated groups $T_1F_0$=0.196*, $T_2F_{24}$=0.191* and $T_3F_{36}$=0.175* respectively when compared with the control NCG=0.163. The result also revealed that increase duration of fasting stress progressively decreased the mean creatinine level as seen in groups $T_2F_{24}$=0.191, $T_3F_{36}$=0.175 and $T_4F_{48}$=0.164.

**Table 3:** Shows the effect of aqueous extract of Vernonia amygdalina on creatinine level (g/dl)

| **Specimens** | **Groups** | | | | |
|---|---|---|---|---|---|
| | **NCG** | **$T_1S_0$** | **$T_2S_{24}$** | **$T_3S_{36}$** | **$T_4S_{48}$** |
| **A** | 0.171 | 0.201 | 0.187 | 0.182 | 0.159 |
| **B** | 0.156 | 0.188 | 0.195 | 0.169 | 0.173 |
| **C** | 0.159 | 0.199 | 0.193 | 0.180 | 0.162 |
| **D** | 0.168 | 0.197 | 0.188 | 0.173 | 0.165 |
| **E** | 0.161 | 0.195 | 0.192 | 0.171 | 0.161 |
| Mean±SD | **0.163±0.006** | **0.196±0.005*** | **0.191±0.003*** | **0.175±0.006*** | **0.164±0.015** |
| Common Mean | | | **0.178** | | |
| p-value | | **<0.00001** | | | |
| f. ratio value | | **41.6426** | | | |

*Statistically significant increase at p<0.05 with respect to control
# Statistically significant decrease at p<0.05 with respect to control

### *3.4 Effect of ABLE administration on Urea concentration*

Table 4 shows the effect of aqueous extract of bitter leaf (ABLE) on Urea concentration (g/dl) of the wistar rats. The results reveal statistically significant (P<0.05) increase in the mean Urea concentration in treatment groups $T_1S_0$=52.1* and $T_2S_{24}$=48.6* respectively and statistically significant decrease (P<0.05) in $T_4S_{48}$=26.0 when compared with the control NCG=36.0. Also, increased duration of fasting stress progressively reduced urea level significantly (P<0.05) as observed in $T_2S_{24}$=48.6, $T_3F_{36}$=36.4 and $T_4S_{48}$= 26.0 respectively.

**Table 4:** Shows the effect of aqueous extract of Vernonia amygdalina on urea level (g/dl)

| **Specimens** | **Groups** | | | | |
|---|---|---|---|---|---|
| | **NCG** | **$T_1S_0$** | **$T_2S_{24}$** | **$T_3S_{36}$** | **$T_4S_{48}$** |
| **A** | 39.0 | 57.3 | 49.2 | 37.4 | 22.0 |
| **B** | 32.0 | 48.7 | 51.7 | 35.8 | 29.0 |
| **C** | 37.0 | 50.4 | 46.8 | 38.2 | 31.0 |
| **D** | 34.0 | 54.3 | 51.1 | 36.1 | 25.0 |
| **E** | 38.0 | 49.9 | 44.2 | 34.5 | 23.0 |
| Mean±SD | **36.0±2.92** | **52.1±3.58*** | **48.6±3.12*** | **36.4±1.44** | **26.0±3.87#** |
| Common Mean | | | **39.82** | | |
| p-value | | **0.00001** | | | |
| f. ratio value | | **57.942** | | | |

*Statistically significant increase at p<0.05 with respect to control
# Statistically significant decrease at p<0.05 with respect to control

## 4. DISCUSSION

This study investigated the effects of V. amygdalina on hematological indices and kidney function in stress induced male Wistar rats. Hematological parameters are important indices in ascertaining the functional status of animals exposed to environmental and physiological changes [9] and the result indicated a marginal increase in WBC counts which was statistically significant in the treated group fasted for 48 hours. It is of note that an increase in WBC count may indicate an immune disorder or infection, however, this increase may be due to the prolonged exposure to fasting stress and this finding agrees with [10] who reported that fasting increased WBC counts in humans during the Ramadan. The reports of [11], [12] also suggested that increase in WBC counts may be attributed to the administration of aqueous extract of V. amygdalina which contains phytochemicals such as sesquiterpenes, saponins, flavonoids, tannins and veernoniosides that allows the animal to respond to infections or serve as anti-nutrients. Hemoglobin concentration is positively correlated with the nutritional status of the animal and increased Hb concentration indicates a better transport and prevents anemia [11]. From the findings of this study, hemoglobin concentration level in the treated non-stressed group ($T_1S_0$) and treated group fasted for 24 hours ($T_2S_{24}$) were significantly increased. The findings also revealed a steady reduction in Hb concentration amongst the treated groups exposed to fasting stress for 24 hours (14.88±0.99g/dl), 38 hours (13.54±2.27g/dl) and 48 hours (12.58±0.19g/dl) respectively. This may be an indication that V. amygdalina may have a potential to increase erythropoietin release from the kidneys, which serves as the humoral regulator of erythrocytes production and also affect the oxygen carrying property of the blood [ 12]. It is of note that hemoglobin is that iron containing element transport metalloprotein within the red blood cells of all vertebrates, and it carries element from the metabolic process organs (lungs) to the remainder of the body wherever it releases the element to oxidize nutrients to produce energy to regulate the functions of the organism [13]. It is suspected that fasting may have a modulating effect on Hb concentration. This finding, is consistent with the reports of [11] who reported that V. amygdalina increases Hb concentration by supporting haematopoietic tissues with production of more erythrocytes and WBC.

Increasing serum creatinine and urea level is an important indicator of poor glomerular filtration and has been a significant clinical marker for renal dysfunction and loss of renal integrity [14]. Our findings revealed that creatinine level is increased by the administration of aqueous extract of V. amygdalina but decreased gradually following the increased duration of fasting stress. Our finding also reveals that urea level was increased by the administration of aqueous extract of V. amygdalina but was progressively reduced as fasting stress duration increased. There was significant reduction in serum urea concentration in group treated with V. amygdalina and fasted for 48 hours, implying that fasting significantly reduces blood urea level. The significant increase in serum creatinine and urea concentration by V. amygdalina may be due to the anti-nutrients present in V amygdalina which may have a compromising effect on the kidney. Creatinine could be a matter of muscle creatin, whose quantity in blood serum is proportional to the body's muscle mass. The number of creatinine is sometimes constant, so elevated levels indicate diminished nephritic operate solely, since it is simply excreted by the kidneys [15]. Gluconeogenesis is sustained by increased proteolysis which releases free glucogenic amino acids circulated in plasma, and are deaminated in the liver with the consequence of increased urea in blood [16]. Our findings on the effect of V amygdalina on the concentration of creatinine and urea in the blood is consistent with the finding of [17] and inconsistent with the reports of [18], [19] who posited that V. amygdalina extract significantly decreased creatinine and urea level in rats thereby offering reno-protective effect. However these contrasting reports, it is imperative for future research to reinvestigate how V. amygdalina plays its role in glomerular filtration function of the kidney nephrons.

## 5. CONCLUSION

This study suggests that aqueous leaf extract of V. amygdalina could improve the haemoglobin concentration, modulate the increase of white blood cell count in response to stress but may compromise the kidneys function as seen by increase in serum creatinine and urea concentration. It also concluded that fasting may play a modulatory role in cushioning the compromising effect V. amygdalina has on the kidney function as evident by its reduction of serum creatinine and urea concentration.

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Conflicts of Interest Statement
We declare that our research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflicts of interest.

Author contributions
IGE, OIC and DCI conceived, designed and implemented the study. UJU, NCC, OIC, EEN, CUJ and DCI contributed to data analysis and interpretation of results. IGE supervised the study. All authors drafted, read, revised and approved the final copy of the manuscript.

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