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In-Vivo Anti-Lipidemia And Anti-Oxidant Activity of *Vernonia Amygdalina Del* (Bitter Leaf) Leaf Extracts Collected In Harmattan on L-Name Induced Hypertensive Male Rats.

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Abstract
This Study Investigated The Antioxidant And Anti-Lipidemia Activity Of Methanolic Extract Of *Vernonia Amygdalina* (VA) Leafs In L-Nitro-Nomega Arginine Methyl Ester (L-NAME) Induced-Hypertensive Rats And Compared With A Standard Hypertensive Drug (Amlodipine).
The Rats Were Firstly Induced With 40mg/Kg Body Weight Of L-NAME For 2weeks Before Being Co-Administered With 5mg/Kg Amlodipine As Well As 100mg/Kg, 200mg/Kg And 400mg/Kg Body Weight Of Extract Respectively For Another 3weeks. Both Administration And Induction Were Done Orally, Once Daily Across Different Groups. The Effects Of The VA Were Then Assessed On Oxidative Stress Markers (Superoxide Dismutase (SOD) And Catalase (CAT) Activities As Well As Reduced Glutathione (GSH) And Malondialdehyde (MDA) Concentrations And Lipid Profile (High Density Lipoprotein Cholesterol (HDL), Low Density Lipoprotein Cholesterol (LDL), Triglyceride (TG) And Total Cholesterol (TC)) In The Serum Of The Rats.
The Extract Was Non Toxic, Having A LD-50 Greater Than 10,000mg/Kg Body Weight And L-NAME Significantly (*P<0.05) Increase The Level Of Blood Pressure In The Induced Groups Causing Hypertension Compared To The Control. Also L-NAME Significantly (*P<0.05) Increased The Levels Of MDA, LDL, TG, And TC While Significantly (*P<0.05) Reducing The Activities Of SOD And CAT And The Concentrations Of GSH And HDL. Treatment With The Extract Significantly (*P<0.05) Reduced The Levels Of MDA, LDL, TG, And TC While Increasing The Activities Of SOD And CAT And Concentrations Of GSH And HDL In The Hypertensive Rats.
This Result Suggest That The Extract Have Potent Antioxidant And Anti-Lipidemia Effect On Hypertensive Rats And Therefore May Possess Antihypertensive Activity

Keywords: *Antioxidant, Anti-Lipidemia, Vernonia Amygdalina, L-Nitro-Nomega Arginine Methyl Ester, Hypertensive Rats, Amlodipine.*

I. Introduction

Phytochemicals Found In Plants Have Been Used To Treat And Cure A Wide Range Of Illnesses. Globally, The Use Of Medicinal Herbs Is Steadily Increasing, The Search For Therapeutic Compounds Derived From

Plant Species Have Increased As A Result Of The Emergence Of Resistant Diseases, Chronic Disorders And The Development Of Scientific Knowledge Regarding Herbal Treatments As Important Therapy Alternatives [1]. Phytotherapy Is Still Widely Used Till Date Despite The Tremendous Advancements In Modern Medicine Because Medicinal Plants Have Long Been Used To Prevent And Treat A Wide Range Of Health Conditions [2]. Also, These Orthodox Drugs Are Mainly Associated With Some Side Effects And Sometimes Expensive, Limiting Its Affordability By The People Living In Low-Income Nations Which Has Also Increase The Dependent Rate Of People On Herbal Medications That Is Believed To Have Better Human Compatibility, Lesser Side Effect And Highly Affordable [3].

One Prominent Member Of Those Medicinal Plants In Tropical Africa Belonging To The Asteraceae Family Is *Vernonia Amygdalina* Del Widely Known As Bitter Leaf In English [4]. It Is A Shrub That Can Grows To A Height Of 2 To 5 Meters, Found In Many Different Ecological Zones Across Africa, And It Is Tolerant Of Drought, Yielding Massive Volumes Of Fodder With The Green Leafs Taste Bitter And Have Distinct Smell [4]. This Plant Is Mostly Used In The Management Of Hypertension In Nigeria Due To Its Reported Phytochemical Constituents Which Includes Flavonoids And Phenolic Compounds [5], [6], [7], [8].

Hypertension Is Described As An Abnormal Rise Of Blood Pressure (BP) [9], It Is Known To Be The Commonest Risk Factor For Cardiovascular Diseases (CVD, Which Include Coronary Artery Disease, Heart Failure, Stroke, Myocardial Infarction, Atrial Fibrillation, And Peripheral Artery Disease), Chronic Kidney Disease (CKD), And Cognitive Impairment, And It Is Considered The World's Leading Cause Of Death And Disability [10].

Two Key Conditions Among Several Contributors That Have Been Implicated In Both Hypertension And Cardiovascular Diseases Are Oxidative Stress And Hyperlipidemia. Oxidative Stress Is A Condition Marked By An Imbalance Between The Generations Of Reactive Oxygen Species (ROS) And The Body's Antioxidant Defenses. In L-Nitro-Arginine Methyl Ester (L-NAME) Induced Hypertensive Rats, Excessive ROS Is Produced Leading To Endothelial Dysfunction, A Critical Early Event In The Cascade Of Vascular Changes That Result In Sustained Hypertension. The Impaired Bioavailability Of Nitric Oxide (NO), A Vasodilator, Due To Oxidative Stress Exacerbates Vasoconstriction And Increases Systemic Vascular Resistance [11]. Oxidative Stress Not Only Promotes Vascular Stiffness And Inflammation But Also Contributes To The Activation Of The Renin-Angiotensin-Aldosterone System (RAAS), Further Increasing Blood Pressure. Additionally, ROS Can Damage Cellular Components Such As Lipids, Proteins, And DNA, Thereby Contributing To Long-Term Vascular And Organ Damage.

The Second Condition Is Hyperlipidemia, Which Is A Key Modifiable Risk Factor For Cardiovascular Diseases (Cvds) [12] And Also Exacerbate Hypertension By Causing Endothelial Dysfunction, Persistent Inflammation, And Arterial Stiffness [13].

Vernonia Amygdalina Leaf Is Being Reported To Contain Phytochemical Constituents That Have Shown Promising Effect In Ameliorating Oxidative Damage And Improving Cardiovascular Health In Experimental Models [14] And So The Antioxidant Properties Of *Vernonia Amygdalina* May Help Counteract The Oxidative Stress Observed In Hypertensive Conditions, Thereby Improving Endothelial Function And Reducing Vascular Resistance [14]. Similarly, Preclinical Research Showed That In Hypercholesterolemia Rats, Extract Administration Of *Vernonia Amygdalina* Dramatically Lowers Total Cholesterol, LDL, And Triglycerides While Increasing HDL [15].

Numerous Factors, Such As The Seasonal Changes, Environmental Conditions And Age And Time Of Collection Of Plants Can Affect The Phytochemical Constituents Produced By Plants Which May Have Effect On Its Biological Activity [4].

Our Recent Unpublished Observation Indicated The Harmattan Season As The Most Ideal Period Of The Year For The Collection Of *Vernonia Amygdalina* Leaf In The Treatment Of Hypertension Through *In Vitro* And *Insilico* Studies On Some Selected Markers Of Hypertension And Oxidative Stress. Therefore, This Study Was Done To Assess The Anti-Hypertension And Antioxidant Activity Of *Vernonia Amygdalina* Leaf Methanol Extracts On LNAME-Induced Hypertensive Male Rat

II. Materials And Methods

A. Chemicals

L-Nitro-Nomega Arginine Methyl Ester, Thiobarbituric Acid (TBA), Methanol And Cholesterol Were Purchase From Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). Amlodipine (Horsham Road, North Wales, Teva USA) Was Purchased From A Local Pharmacy Store In Ogbomoso, Nigeria. Diagnostic Kits For Triglyceride, Cholesterol Were Purchased From Boehringer Mannheim Gmbh Diagnostica, Germany. All Other Chemicals And Reagent Were Of Analytical Grade.

B. Preparation Of Plant Extract

Fresh Leaf Sample Of *Vernonia Amygdalina* Was Collected During Harmattan Months (December, January, February) Of The Year 2024/2025 From A Farm In Ladoke Akintola University Of Technology (LAUTECH) Ogbomoso, Latitude N 8^{o} And Longitude E 4^{o} Nigeria. The Sample Was Identified And Authenticated With A Registered Voucher Number LHO 899 At The Herbarium Of The Department Of Pure And Applied Biology, LAUTECH. Subsequently, It Was Air-Dried For 14 Days And Then Pulverized To Fine Particle Size And Extracted With Methanol Using Cold Extraction Method For 72 Hours At Room Temperature. The Methanolic Extract Was Then Filtered Using Whatman No1 Filter Paper. The Filtrate Was Concentrated With Rotary Evaporator At 35°C To Obtain Gelly-Like Extract Which Was Then Weighed And Stored In An Airtight Container Inside A Refrigerator For Further Analysis [15] With Slight Modification. The Percentage Yield Of The Extracts Was Calculated As Follows:

Percentage Yield (%) =Weight Of Crude Extract/Weight Of Starting Plant Material *100

Where:

Weight Of Crude Extract=Weight Of *Vernonia Amygdalina* Extract Collected In Harmattan

Weight Of Starting Material=Weight Of Dried *Vernonia Amygdalina* Leaf Powder.

C. Ethical Approval And Animal Welfare

The Study Protocol Was Approved By The Institutional Ethical Committee Of Animals In Research, Ladoke Akintola University Of Technology (Lautech), Ogbomoso Nigeria. All Rats Were Procured From The Department Of Physiology And Housed In The Departmental Animal Facility. The Animals Were Given Two Weeks Of Acclimatization Period In Wire-Floored Cages Under 12/12 Light/Dark Cycle At 25^{0}C, $43 \pm 10\%$ Relative Humidity And Allowed Free Access To Water And Rat-Chow.

D. Animals

Exactly 48 Adult Male Wistar Rats Weighing 140–180g Were Used For The Study, 12 Of Them Were Used For The Acute Toxicity Test While The Remaining 36 Rats Were Used For The Experimental Design.

E. Acute Toxicity Study

The Rats Were Divided Into 3 Groups (N=4) [16]. The Extract Was Dissolved In Distilled Water And Administered Orally Once Daily On Three Different Doses Which Are 2500mg/Kg Body Weight Of Rat, 5000mg/Kg Body Weight Of Rat And 10000mg/Kg Body Weight Of Rat [17] And They Were Observed For Signs Of Toxicity For 7days [16], Which Include; Lethargy, Tremors And Diarrhea [17]. Also, The Number Of Deaths Were Recorded And The LD50 Was Calculated According To Method OECD, [16] With Slight Modification.

F. Experimental Design

The Rats Were Randomly Divided Into 6 Groups A-F (N=6). For The First 2 Weeks Group A, B, D, E And F Were Being Induced Orally With 40% L-NAME While Group C (Control) Was Given Ratchow And Water Only. Thereafter The Weight And Blood Pressure Of The Rats Were Measured And Recorded As The First Week. It Was Found That All The Animals In Group A, B, D, E, F Have Developed Hypertension In Relative To Group C.

For Another 3 Weeks The Animals Were Being Induced And Treated As Follows Group A Was Allowed To Take Rat-Chow And Water And Administered With A Standard Drug 25mg/Kg Body Weight Of Extract (Amlodipine) Concurrently With 40%L-NAME, Group B Was Given Rat-Chow And Water With 200mg Per Kg Body Weight Of Extract Concurrently With 40%L-NAME, Group C (Postive Control) Was Fed With Rat-Chow And Drinking Water Only, While Group D And F Were Administered With 200mg And 400mg Per Kg Body Weight Of The Extract Respectively Concurrently With 40%L-NAME, Group E (Negative Control) Was Induced With 40%L-NAME Without Any Treatment, With Free Allowance To Rat-Chow And

Water. Induction And Administration Were Done Orally, Once Daily For 3 Weeks Using Oral Cannula With The Weight Of Animals Taken Once A Week. On The 21st Day Of Administration, The Animals Were Made To Fast Overnight And Sacrificed Using Cervical Dislocation The Next Day. The Blood Of The Rats Were Collected For The Analysis Of Lipid Profile And Oxidative Stress. The Blood Was Centrifuge At 3000rpm For 15min And The Serum Was Collected For The Both Analyses.

G. Serum Lipid Profile Assay

The Collected Serum Samples Were Analyzed For Lipid Profile. High-Density Lipoproteincholesterol (HDL-C) Was Assayed Using An Assay Kit. Triglyceride (TG) Content Was Evaluated By Enzymatic Method Using An Assay Kit. Total Cholesterol (TC) Was Determined According To The Method Of Parakh And Jank [18] . Low-Density Lipoprotein Cholesterol (LDL-C) Was Calculated According To Friedwald *Et Al.* [19].

H. Oxidative Stress Markers Analysis

Standard Biochemical Techniques Were Used To Measure The Activities Of Superoxide Dismutase (SOD), Catalase (CAT), And Concentrations Of Reduced Glutathione (GSH) And Malondialdehyde (MDA) As Follows

I. Superoxide Dismutase (SOD) Activity

The SOD Activity Was Determined By The Method Reported By Misra And Fridovich, [20] . Briefly, The Serum (0.5 Ml) Was Diluted With 0.5 Ml Of Distilled Water. The Ice-Cold Ethanol (0.25 Ml) And Chloroform (0.15 Ml) Was Mixed With Diluted Serum And Centrifuged At 2500 Rpm At 4 °C For 15 Min. Carbonate Buffer (1.5 Ml) And Ethylenediamine Tetra Acetic Acid (EDTA) Solution (0.5 Ml) Were Added To 0.5 Ml Supernatant. The Reaction Was Initiated By The Addition Of 0.4 Ml Of Epinephrine And The Change In Optical Density/Min Was Measured At 480 Nm Against Blank. The SOD Activity Was Expressed As U/Mg Protein [20] .

Ii. Catalase (CAT) Activity

CAT Activity Was Determined By The Method Reported By [21]. Briefly, The Serum (0.1 Ml) Was Added To Cuvette Containing 1.9 Ml Of 50 Mm Phosphate Buffer (Ph 7.0). The Reaction Was Initiated By The Addition Of 1.0 Ml Of Freshly Prepared 30 Mm H_2O_2 Solution. The Rate Of Decomposition Of H_2O_2 Was Measured Spectrophotometrically With The Absorbance At 240 Nm. The First Absorbance (A1) Was Read After 15 S (T1) And The Second Absorbance (A2) Was Read After 30 S (T2). The Activity Of CAT Was Expressed As U/Mg Protein [21].

Iii. GSH Content

GSH Content Was Determined By The Method Reported By [22]. Briefly, Equal Volumes Of Serum And 20% TCA Was Mixed. The Precipitated Fraction Was Centrifuged At 2500 Rpm At 4 °C For 15 Min. Then 2.0 Ml Of DTNB [5, 5′ -Dithiobis-(2-Nitrobenzoic Acid)] Reagent. Was Added To 0.25 Ml Of Supernatant. The Final Volume Was Made Up To 3.0 Ml With Phosphate Buffer. The Absorbance Was Recorded At 412 Nm Against Reagent Blank. The Amount Of GSH Was Expressed As Nmol Of GSH/Mg Protein [22].

Iv. MDA Concentration

MDA Level In The Serum Was Estimated By The Method Reported By Wang *Et Al.,* [23]. Briefly The Serum (2.0 Ml) Was Added To 2.0 Ml Of Freshly Prepared 10% W/V Tricholoroacetic Acid (TCA) And The Mixture Was Kept In An Ice Bath For 15 Min, The Precipitate Formed Was Separated By Centrifugation At 2500 Rpm At 4 °C For 15 Min. Clear Supernatant Solution (2 Ml) Was Mixed With 2.0 Ml Of Freshly Prepared Thiobarbituric Acid (TBA). The Resulting Solution Was Heated In A Water Bath For 10 Min At 80 °C And Then Immediately Cooled In An Ice Bath For 5 Min. The Color Developed Was Measured At 532 Nm Against Blank. The Value Was Expressed As Nmol Of MDA/Mg Protein [23].

I. Statistical Analysis

Statistical Analysis Was Performed Using The SPSS 19.0 Software (SPSS, Inc., Chicago, IL, USA). The Data Was Presented As The Mean ± Standard Error Of Mean. The Differences Among The Variables Of The

Different Groups Were Evaluated By One-Way Analysis Of Variance Followed By A Post Hoc Student-Newmann-Keuls Test. Student'S T-Test Was Used When Only Two Sets Of Data Were Compared, While X2 And Fisher's Exact Tests Was Used In The Comparison Of Categorical Data As Required. *P<0.05 Was Considered To Indicate A Statistically Significant Difference [24] With Slight Modification.

III. RESULTS

A. Percentage Yeild Of *Vernonia Amygdalina* Extract Collected In Harmattan.

The Percentage Yeild Of The Extract Was 13.33%

B. Toxicity Level Of The Extract Of *Vernonia Amygdalina* Collected In Harmattan.

The Extract Was Non Toxic As It Has An LD-50 Greater Than 10,000mg/Kg Body Weight

C. Blood Pressure Of The Experimental Animal After Induction Of Hypertension

Table 1 Depicted The Effect Of L-NAME Induction On Blood Pressure Of The Experimental Rat After Two Weeks. The Groups A, B, D, E& F Showed Significant Increase (*P<0.05) In The Level Of Systolic Blood Pressure, Diastolic Blood Pressure And Mean Arterial Blood Pressure Compared To Group Which Only Received Feed And Water During This 2weeks

Table 1: Effect Of L-NAME Induction On Blood Pressure Of The Experimental Rats After Two Weeks.

Group	SBP(Mmhg)	DBP(Mmhg)	MBP(Mmhg)
A	**162.00 ±0.63**	**102.00±0.53**	**132.00±1.26**
B	**177.50±0.63**	**105.00±2.24**	**141.00±1.58**
C	**108.50±0.63**	**74.50±1.26**	**91.50±0.67**
D	**167.00±2.03**	**101.00±0.77**	**134.00±1.26**
E	**162.50±0.63**	**98.50±1.68**	**130.50±0.62**
F	**164.00±1.02**	**104.00±0.71**	**134±1.26**

Values Expressed As Mean ± Standard Error Of Mean, Where; SBP Is Systolic Blood Pressure, DBP Is Diastolic Blood Pressure, MBP Mean Blood Pressure.

D. Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Body Weight Of Hypertensive Rats.

Table 2 Showed The Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Body Weight Of Hypertensive Rats. It Was Observed That There Was No Significant Different (*P<0.05) On The Body Weight Of Group A, B, D, And F, Which Are The Administered Groups Compare To Group C And E Which Are The Positive And Negative Control Groups Respectively.

Table 2: Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Body Weight Of Hypertensive Rats.

Group	Week 1(G)	Week 2(G)	Week 3(G)	Week 4(G)
A	**147.50±0.57**	**154.80±1.09**	**163.20±1.08**	**178.20±0.89**
B	**162.30±0.62**	**165.00±1.32**	**168.10±1.84**	**178.30±0.91**
C	**185.50±0.98**	**187.80±0.95**	**194.50±1.18**	**200.20±1.29**
D	**159.30±1.57**	**162.00±0.63**	**165.00±1.68**	**168.30±1.68**
E	**160.30±0.68**	**165.40±1.30**	**167.20±2.03**	**170.30±1.02**
F	**170.10±1.08**	**173.20±0.63**	**175.00±0.98**	**180.10±0.84**

E. Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Superoxide Dismutase (SOD) Activity Of Hypertensive Rats.

Figure 1 Revealed The Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Superoxide Dismutase (SOD) Activity Of Hypertensive Rats. It Was Observed That, The Activity Of SOD Was

Significantly (*P<0.05) Reduced In The L-NAME Induced Group Compare To The Positive Control Group, Administration Of The Extracts Significantly (*P<0.05) Increased The Activity Of SOD To Near Normal, Administration Of The Extracts Also Resulted In Increased Activity Of SOD Compared To Amlodipine. The Corrective Effect Of The Extract On SOD Activity Was In Dose Dependent Manner With The 400mg/Kg Body Weight Extract Resulting In Higher SOD Activity Compared To The Positive Control Group

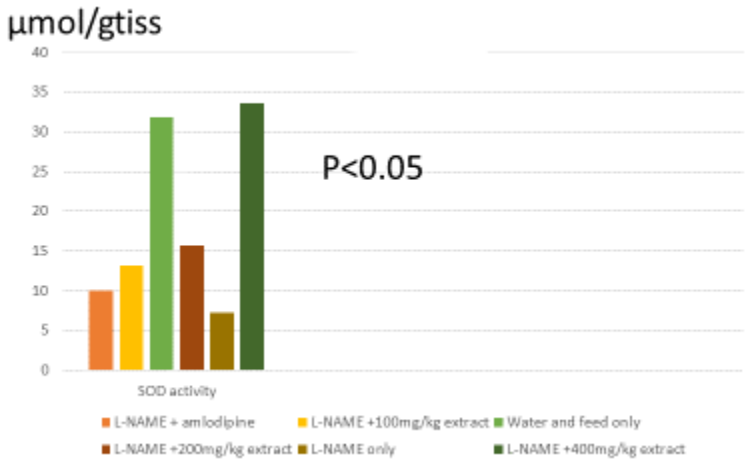

Figure 1: Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Superoxide Dismutase (SOD) Activity Of Hypertensive Rats.

Where; SOD Means Superoxide Dismutase, LNAME Means L-Nitro-Nomega Arginine Methyl Ester, Extract Means *Vernonia Amygdalina* Leaf Extract.
Values Express As Mean ± Standard Error Of Mean P Value Of (*P<0.05) Considered Significant

F. Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Catalase (CAT) Activity Of Hypertensive Rats.

Figure 2 Depicted The Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Catalase (CAT) Activity Of Hypertensive Rats. It Was Observed That, L-NAME Significantly (*P<0.05) Reduced The Activity Of CAT In Rats As Compared To The Positive Control Group, Administration Of The Extracts Significantly (*P<0.05) Increased The Activity Of CAT In The Induced Group, The Extracts Also Showed Higher Ameliorative Potential In Improving The Activity Of CAT And Restoreing It To Normal Compared To Amlodipine.

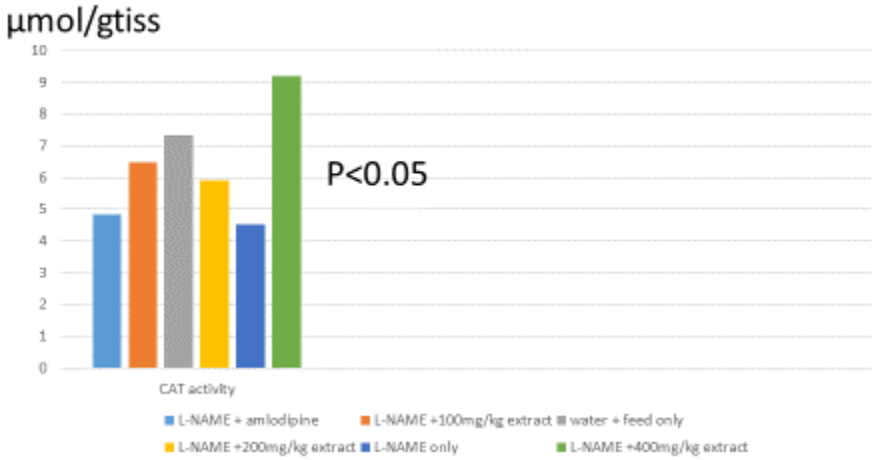

Figure 2: Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Catalase (CAT) Activity Of Hypertensive Rats.

Where; CAT Means Catalase, LNAME Means L-Nitro-Nomega Arginine Methyl Ester, Extract Means *Vernonia Amygdalina* Leaf Extract.
Values Express As Mean ± Standard Error Of Mean P Value Of (*P<0.05) Considered Significant

G. Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Reduced Glutathione (GSH) Concentration Of Hypertensive Rats.

Figure 3 Showed The Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Reduced Glutathione (GSH) Concentration Of Hypertensive Rats. It Was Found That, L-NAME Induction Causes A Significant (*P<0.05) Reduction In The Concentration Of GSH In The Serum Of The Rats Compared To The Positive Control Group, Administration Of The Extracts Significantly (*P<0.05) Restored The Decreased Concentration Of GSH In The Induced Groups To Near Normal, The Extracts Also Compete Favourably In Terms Of Efficacy Compared To Amlodipine. The Ameliorative Effect Of The Extract In Restoring The GSH Concentration Was In Dose Dependent Manner.

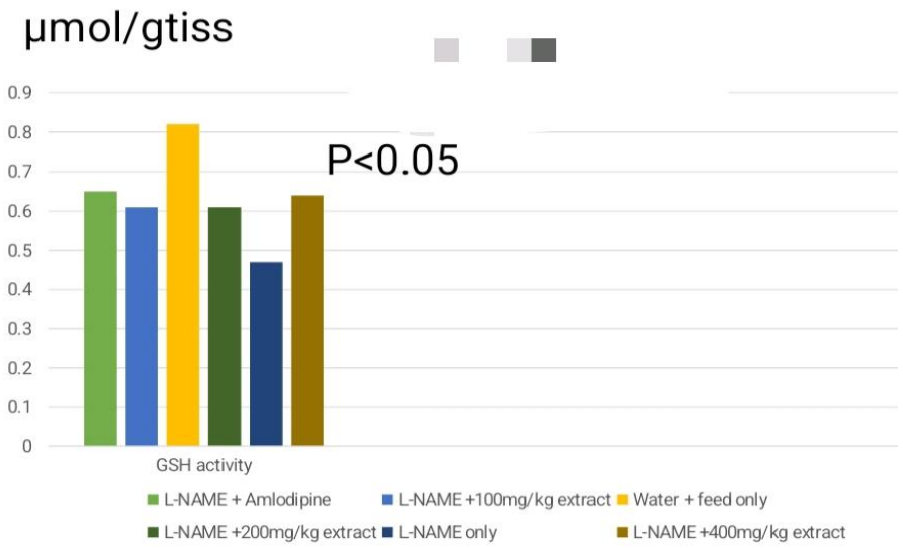

Figure 3: Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Reduced Glutathione (GSH) Concentration Of Hypertensive Rats.

Where; GSH Means Reduced Glutathione, LNAME Means L-Nitro-Nomega Arginine Methyl Ester, Extract Means *Vernonia Amygdalina* Leaf Extract.
Values Express As Mean ± Standard Error Of Mean P Value Of (*P<0.05) Considered Significant

H. Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Malondialdehyde (MDA) Level Of Hypertensive Rats.

Figure 4 Depicted The Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Malondialdehyde (MDA) Level Of Hypertensive Rats. As Shown, L-NAME Significantly (*P<0.05) Elevated The Level Of MDA In Rats As Compared To The Positive Control, Administration Of The Extracts At 100 And 200mg/Kg Body Weight Significantly (*P<0.05) Reduced The High Level Of MDA In The Induced Groups To Near Normal, The Extracts At 100 And 200mg/Kg Also Showed A Better Corrective Effect On MDA Level Compared To Amlodipine. However, It Was Found That There Was No Significant (*P<0.05) Difference Between The L-NAME Induced Group, Amlodipine Administratered - L-NAME Induced Group And The 400mg/Kg Body Weight Extract Administratered - L-NAME Group.

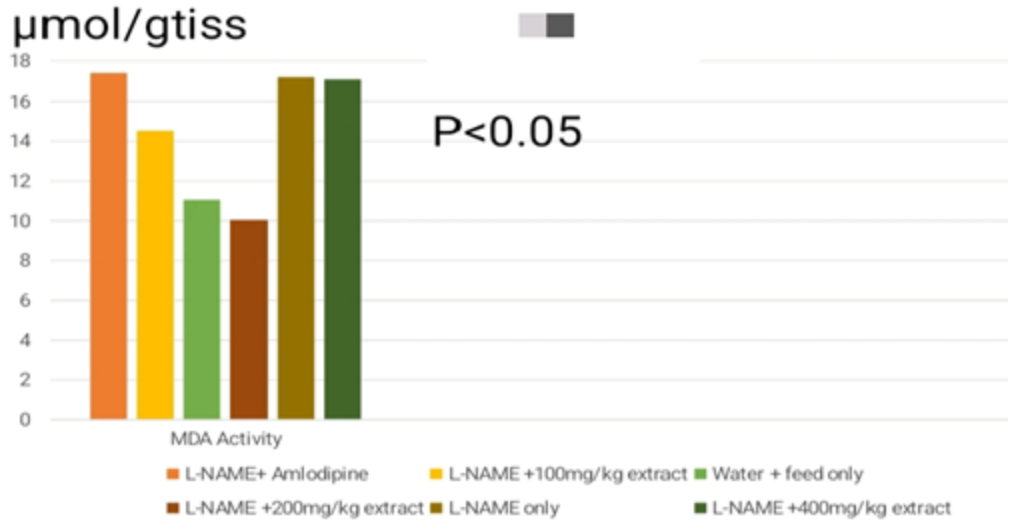

Figure 4: Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Malondialdehyde (MDA) Level Of Hypertensive Rats.

Where; MDA Means Malondialdehyde, LNAME Means L-Nitro-Nomega Arginine Methyl Ester, Extract Means *Vernonia Amygdalina* Leaf Extract.
Values Express As Mean ± Standard Error Of Mean P Value Of (*P<0.05) Considered Significant

I. Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On High Density Lipoprotein Cholesterol (HDL) Level Of Hypertensive Rats.

Figure 5 Revealed The Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On High Density Lipoprotein Cholesterol (HDL) Level Of Hypertensive Rats. L-NAME Significantly (*P<0.05) Reduced The Level Of HDL In Rats, Administration Of The Extracts Significantly (*P<0.05) Restored The Level Of HDL To Normal, The Extracts Also Showed Better Efficacy Compared To Amlodipine. The Corrective Effect Of The Extract In Restoring The Reduced HDL Level Is In Dose Dependent Manner.

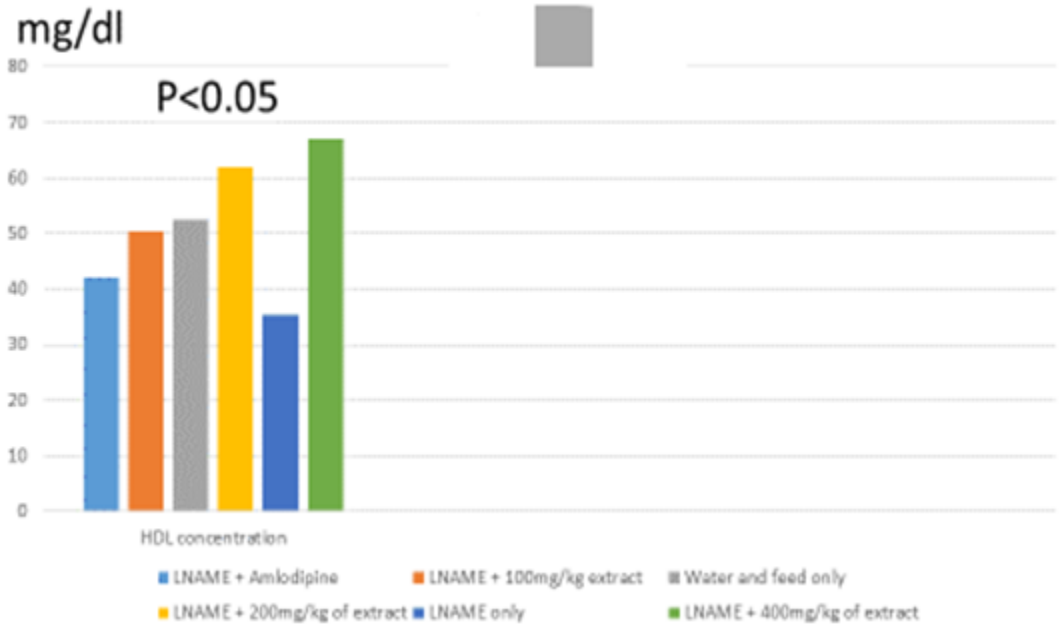

Figure 5: Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On High Density Lipoprotein Cholesterol (HDL) Level Of Hypertensive Rats.

Where; HDL Means High Density Lipoprotein Cholesterol, LNAME Means L-Nitro-Nomega Arginine Methyl Ester, Extract Means *Vernonia Amygdalina* Leaf Extract.

Values Express As Mean ± Standard Error Of Mean P Value Of (*P<0.05) Considered Significant

J. Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Low Density Lipoprotein Cholesterol (LDL) Level Of Hypertensive Rats.

Figure 6 Showed The Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Low Density Lipoprotein Cholesterol (LDL) Level Of Hypertensive Rats. As Observed, L-NAME Significantly (*P<0.05) Increased The Level Of LDL In Rats, Administration Of The Extracts Significantly (*P<0.05) Reduced The Elevated Level Of LDL To Near Normal, The Extracts Also Compete Favourably In Terms Of Efficacy Compared To Amlodipine. Meanwhile, The Extract Showed It's Highest Efficacy At 200mg/Kg Body Weight.

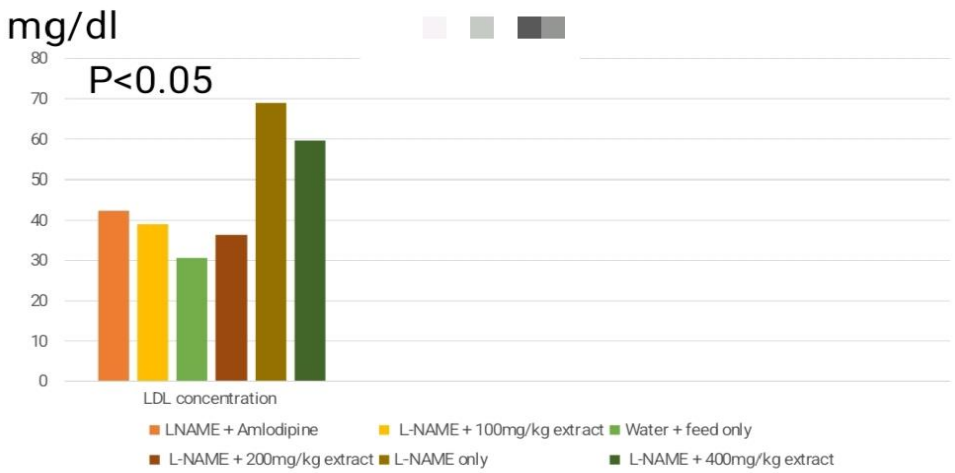

Figure 6: Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Low Density Lipoprotein Cholesterol (LDL) Level Of Hypertensive Rats.

Where; LDL Means Low Density Lipoprotein Cholesterol, LNAME Means L-Nitro-Nomega Arginine Methyl Ester, Extract Means *Vernonia Amygdalina* Leaf Extract.

Values Express As Mean ± Standard Error Of Mean P Value Of (*P<0.05) Considered Significant

K. Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Triglyceride (TG) Level Of Hypertensive Rats.

Figure 7 Depicted The Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Triglyceride (TG) Level Of Hypertensive Rats. It Was Observed That L-NAME Significantly (*P<0.05) Increased The Level Of TG In Rats, Administration Of The Extracts Significantly (*P<0.05) Reduced The High Level Of TG To Near Normal, The Extracts Also Showed A Better Efficacy Compared To Amlodipine. Meanwhile, The Extract Showed It's Highest Efficacy In Normalizing The Level Of TG At 200mg/Kg Body Weight.

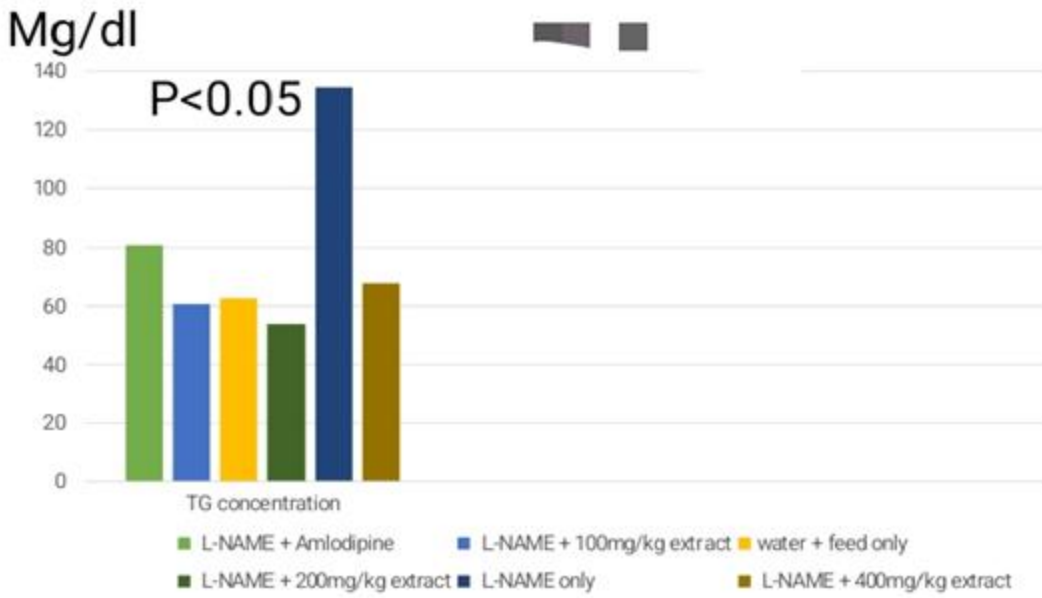

Figure 7: Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Triglyceride (TG) Level Of Hypertensive Rats.

Where; TG Means Triglyceride, LNAME Means L-Nitro-Nomega Arginine Methyl Ester, Extract Means *Vernonia Amygdalina* Leaf Extract.
Values Express As Mean ± Standard Error Of Mean P Value Of (*P<0.05) Considered Significant
L. Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Total Cholesterol (TC) Level Of Hypertensive Rats.
Figure 8 Revealed The Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Total Cholesterol (TC) Level Of Hypertensive Rats. As Shown, L-NAME Significantly (*P<0.05) Elevated The Level Of TC In Rats, Administration Of The Extracts Significantly (*P<0.05) Decreased The High Level Of TC To Near Normal, The Extracts Also Compete Favourably In Terms Of Efficacy Compared To Amlodipine. Meanwhile, The Extract Showed It's Highest Efficacy In Normalizing The Level Of TC At 200mg/Kg Body Weight, Although, This Was Not Significant (*P<0.05) Compare To The Extract At 400mg/Kg Body Weight Concentration.

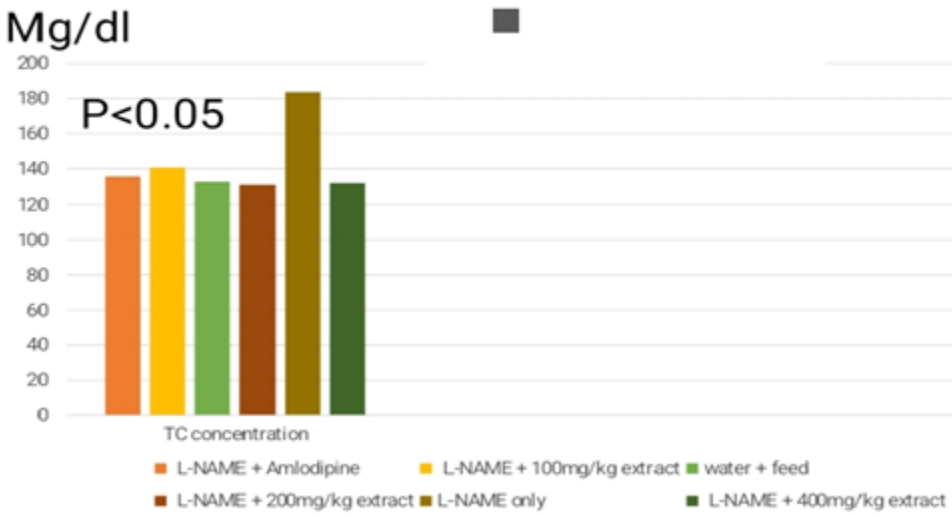

Figure 8: Effect Of *Vernonia Amygdalina* Leaf Extract Collected In Harmattan On Total Cholesterol (TC) Level Of Hypertensive Rats.

Where; TC Means Total Cholesterol, LNAME Means L-Nitro-Nomega Arginine Methyl Ester, Extract Means *Vernonia Amygdalina* Leaf Extract.
Values Express As Mean ± Standard Error Of Mean P Value Of (*P<0.05) Considered Significant

IV. Discussion

The Antihypertensive Effect Of *Vernonia Amygdalina* Del Leaf Has Been Documented In Several Invitro And Invivo Studies [5], [6], [7], [8]. These Antihypertensive Studies Were In Most Cases Assessed In Collaboration With Their Antioxidant And Anti-Lipidemic Effect. The Biological Activity Of A Plant Is Mostly Dependent On The Diverse Array Of Phytochemical Constituents Present In The Plant, And In The Case Of *Vernonia Amygdalina* Some Of The Factors That Has Been Implicated Were; The Choice Of Solvent Used For The Extraction And Various Environmental Factors [4], [25], [26].

In Terms Of Solvent Used For The Extraction Suliamon Et Al., [25] Reported That The Methanolic Leaf Extract Yielded Higher Concentration Of Bioactive Compounds In Most Cases Compared To The Aqueous Extract Using Gas Chromatography-Mass Spectrometry (GC-MS) And High Performance Liquid Chromatography Techniques (HPLC), It's Similarly Reported That The Methanolic Extract Have Higher Radical Scavenging Activity On DPPH And ABTS At All Dosages Considered Suggesting For It Better Antioxidant Capacity. Abiola Et Al., [26] Also Reported That Alcoholic Solvents Such As Methanol And Ethanol Can Be Used To Extract More Lipophilic Constituents, Especially Phenolic Compounds Better Than Aqueous Solvent. These Among Some Other Reported Studies Necessitated The Choice Of Methanolic Extract Used In This Study. Environmental Factor Such As Geographical Location, Seasonal Changes, And Soil Factors Is Another Factor Contributing To The Diversity In The Distribution Of Bioactive Compounds In *Vernonia Amygdalina* [4].

Our Recent Unpublished Observation Concluded That The Methanolic Extract Of *Vernonia Amygdalina* Collected During Harmattan Season Have The Highest Antioxidant Property Compared To Those Collected At Other Seasons. It Was Found That The Extract Used In This Study Was Non-Toxic, Having An LD-50 Greater Than 10,000mg/Kg Body Weight In Rat, A Result Similar To What Was Reported By Abiola Et Al. [26].

In Rats, L-NAME Causes An Irreversible Inhibition Of Nitric Oxide (NO) Synthase, Leading To Decreased Nitric Oxide Bioavalability And Increased Angiotensin (ANG) II Production And Vasoconstriction Causing Increased Blood Pressure. ANG II Causes The Upregulation Of NADPH Oxidase, Which Is The Primary Producer Of Superoxide In Hypertensive Individuals [27]. This Superoxide Binds To The Available NO, Leading To Peroxinitrite And This Peroxinitrite Can Also Oxidises Nitric Oxide Synthase To Produce More Superoxide [28]. Furthermore Superoxide Can Also Oxidize Tetrahydrobiopterin (BH_4) And This Oxidized BH_4 Form Causes The Uncoupling Of NOS Generating More Superoxide. Other Generators Of Reactive Oxygen Species In Hypertensive Individual Are Xanthine Oxidase And Some Mitochondrial Enzymes All These Explaining The Role Of Oxidative Stress In The Development And Progression Of Hypertension Suggesting Antioxidant Therapy As Measured To Reduced Blood Pressure And Oxidative Damage Associated With Cvds Due To Their Ability To Terminate The Chain Reactions Of Reactive Oxygen Species, Removing Free Radicals And Inhibiting Other Oxidation Reactions [29].

The High Increase In The Activity Of Superoxide Dismutase And Catalase Which Are Enzymatic Antioxidant As Well As The Increase In Concentration Of GSH; A Non Enzymatic Antioxidant Observed In The *Vernonia Amygdalina* Treated Groups May Be Due To The Antioxidant Property Of *Vernonia Amygdalina* Bioactive Compounds Which Have Been Discussed In Several Studies [30], [31], [32]. These May Be As A Result Of The High Level Of Flavonoids, Polyphenols, And Vitamin C And E, Which Are Powerful Antioxidants And Have All Been Reported To Be Present In High Quantities In *Vernonia Amygdalina* [33] Similarly, In The Treated Groups With *Vernonia Amygdalina*, The Reduction In The Level Of MDA Also Explains The Role Of These Bioactive Compounds, Particularly Flavonoids And Phenolic Compounds [33].

This Result Was In Line With A Similar Study On Oxidative Stress Markers In Hypertensive Rat By Onyema-Iloh Et Al. [34] Although Nacl Was Used To Induced Hypertension In The Study. Abiola Et Al. [26]

Also Recorded An Increment In The Activity Of Antioxidant Enzyme In Monosodium Glutamate (MSG) Induced Rat Treated With Aqueous Extract Of *Vernonia Amygdalina*

Also, Some Studies Have Reported That Drugs Or Herbal Remedies Use In The Management Of Hyperlipidemia Can Reduce Hypertension And Its Cvds Associated Morbidity And Mortality [35], Therefore The Focus Of Many Extensive Studies Have Shifted To The Therapeutic Benefit Associated With Medicinal Plants In The Management Of Cvds Focusing On Hyperlipidemia [36]. In This Study, The Lipid Lowering Effect Of *Vernonia Amygdalina* On Rat Induced With L-NAME Was Investigated For A Period Of 3 Weeks After A Significant High Blood Pressure Has Developed. As Observed, The Significant Increase In The Levels Of LDL, TG, And TC And Decrease In HDL In The Induced Group Compared To The Control May Be As A Result Of Atherosclerosis Plaque In The Blood Vessels Of Hypertensive Rat Which Can Also Increase Vasoconstriction [29], This Result Is In Line With The Previous Findings [15; 37]. However, *Vernonia Amygdalina* At Different Dosages Was Able To Significantly Reverse This Hyperlipidemia By Increasing The Level Of HDL And Reducing The Levels Of LDL, TG, And TC Compare To The L-NAME Induced Group Only Which Was In Line With Previous Studies. For Instance, Nwanjo. [38], Reported That *Vernonia Amygdalina* Significantly Reduced Hepatic TG And LDL Cholesterol Levels In Streptozotosin Induced Diabetic Rat. Also, Adaramoye *Et Al.* [15], Reported Low Levels Of LDL, TG, And TC In Rat Fed With Cholesterol After 9 Weeks Of Administration With *Vernonia Amygdalina*. Epidemiological And Clinical Findings Have Established An Association Between Low Levels Of HDL Cholesterol And Increased Risk Of Cardiovascular Diseases [39] Necessitating The Need For Therapeutic Agents That Can Raise The Level Of HDL Cholesterol. The High Level Of HDL Cholesterol In The *Vernonia Amygdalina* Treated Groups Compared To The Other Induced Groups Which Was Also Inline With Previous Study [15] May Be As A Result Of Some Bioactive Compounds Present In The Extract That Have Hypolipidemic Properties. HDL Can Counteract LDL Oxidation And Also Promote The Reverse Cholesterol Pathway Inducing An Eflux Excess Accumulated Cellular Cholesterol And Prevent The Generation Of An Oxidatively Modified LDL Cholesterol [37].

Finally, The Relationship Between Oxidative Stress And Hyperlipidemia Found In This Study On Hypertensive Rat Is Also In Line With The Previous Study Of Adaramoye Et Al. [15] Who Reported A Positive Association Between Oxidative Stress, Hypercholesterolemia And Risk Of Cvds In Rat Induced With Cholesterol. Although, More Studies Are Needed To Unravel The Exact Mechanism Through Which This Occur. However, This Study Was Able To Establish That *Vernonia Amygdalina* Leaf Methanol Extract Collected During Harmattan Season Could Be A Strong Therapeutic Agent In The Treatment Of Hyperlipidemia And In Reversing High Level Of Oxidative Stress In Hypertensive Individuals And Inturn May Be A Strong Candidate In The Management Of Hypertension.

V. Conclusion

Findings From This Study Confirms The Anti-Lipidemic Effects Of *Vernonia Amygdalina* Collected In Harmattan Season And Its Corrective Effect In Reversing The Elevated Oxidative Stress Level In Hypertensive Rats Occurred As A Result Of The Induction Of L-NAME. This Extract At 200 And 400mg/Kg Were Effective In Normalising The Serum Lipid Profile Of The Rat And Counteracting The High Level Of Oxidative Stress In The Rat To Near Normal. More Researches Are Needed To Identify The Exact Component(S) In *Vernonia Amygdalina* During This Season Responsible For The Ammelerative Efficacy As Such Component(S) May Be A Potential Drug Candidate In Managing Hypertension.

VI. Conflict Of Interest

Non Declared.

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