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Renoprotective Effects of Bitter Leaf (*Vernonia Amygdalina*) Extracts on Metabolic Syndrome in Albino Wistar Rats

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Abstract

Chronic Kidney Disease (CKD) is still a major problem of modern clinical medicine. It has been noted that there is an existing link between all forms of chronic and acute kidney injury, and increased effects of metabolic syndrome, during injury progression. This present study evaluates the renoprotective effects of bitter leaf extracts in metabolic syndrome in rats. Twenty (20) albino rats were grouped into A-D and received the following treatments on a daily basis for 4 weeks; for group A (negative control), the rats received oral administration of Ghee + high cholesterol diet (HCD) + sucrose solution (SS) without treatment. Group B (low dose group) received Ghee + HCD + SS + Low dose MEVA (200mg/kg, oral). Group C (high dose group) received Ghee + HCD + SS + High dose MEVA (400mg/kg, oral). Group D which is the normal group received no form of intoxicant or treatment. Kidney injury was assessed through biochemical analyses of serum creatinine, blood urea nitrogen (BUN), Na+ and K+ assay. Data analysis was done using GraphPad prism version 7.0 and results from biochemical analysis were done using mean ± SEM. The level of significance was tested using one-way analysis of variance (ANOVA), followed by the Turkey post hoc analysis and the probability levels <0.05 were considered significant. Administration of Ghee + HCD + SS caused an increase in renal biochemical parameters, thus: BUN (37.92 ± 4.21 mg/dl), Creatinine (1.62 ± 0.04 mg/dl), K+ (7.68 ± 0.09 mmol/l), Na^+ (135.41 ± 0.23 mmol/l). Oral administration of low dose of MEVA improved the kidney function as seen through the following results: BUN (21.75 ± 3.48 mg/dl), Creatinine (1.04 ± 0.05 mg/dl), K^+ (5.92 ± 0.24 mmol/l), Na^+ (140.02 ± 3.10 mmol/l). Administration of a high dose MEVA greatly improved the kidney function as thus: BUN (21.68 ± 1.09 mg/dl), Creatinine (0.92 ± 0.19 mg/dl), K^+ (6.19 ± 0.76 mmol/l), Na^+ (143.88 ± 0.35 mmol/l). The study reveals that bitter leaf (*Vernonia amygdalina*) extracts have a renoprotective effect in metabolic syndrome.

Keywords: High Fat Diet, High Sugar Diet, Metabolic Syndrome, Kidney, Vernonia Amygdalina

Introduction

Metabolic syndrome (MetS) is otherwise known as syndrome X, insulin resistance syndrome, Reaven syndrome, and "the deadly quartet". It is a name given to the combination of disorders such as central obesity and abdominal obesity, systemic hypertension, insulin resistance (or type 2 diabetes mellitus), and atherogenic dyslipidaemia (specifically hypertriglyceridemia

and reduced levels of high-density lipoprotein cholesterol) [1]. The risk factors for the development of MetS include but are not limited to: Positive family history, smoking, increase in age, obesity, physical inactivity, sugary drink and soft drink consumption, excessive intake of alcohol, unhealthy dietary patterns, regular use of antipsychotic drugs (e.g. clozapine).

Current therapeutic options for metabolic syndrome in individuals are limited to treatment for hypertension, hyperglycaemia, and hypertriglyceridemia, as well as dietary control measures and regular exercise [1]. However, traditional alternatives such as the African bitter leaf (Vernonia amygdalina) has been acclaimed to have a healing effect on the different conditions pertaining to metabolic syndrome (obesity, hypertension, insulin resistance, and dyslipidaemia). This, therefore, is the basis of this research, which is aimed to study the renoprotective effects of extracts of bitter leaf (*Vernonia amygdalina)* on animal models of metabolic syndrome in rats.

Bitter leaf is a tiny plant in the Asteraceae family with dark green leaves and rough bark. They are native to tropical Africa, but they are primarily farmed in West Africa, where they thrive in a variety of soils [2]. The leaves contain phenolic compounds, flavonoids, alkaloids, saponins, sesquiterpene lactones, and many other bioactive chemicals. It's a well-known antioxidant [3]. Antioxidants protect cells from oxidative stress and aid in the prevention of metabolic illnesses such as diabetes, cancer, and heart disease. According to studies, the chemicals found in the leaves contribute to the bitter leaf's amazing health advantages.

A large number of studies have confirmed that metabolic syndrome can lead to changes in renal structure and function, such as a decreased glomerular filtration rate (GFR) and increased urinary microalbumin [4]. There are different biochemical parameters to determine the extent to which metabolic syndrome affects the kidney;

Urea is the major nitrogenous outcome of metabolic breakdown of protein in humans. It is dissolved within the blood and transported and excreted by the kidney as a component of urine [5]. Creatinine is the breakdown product of phosphocreatine released from striated muscle at a gentle state. It is filtered by the glomerulus and a little amount is equally secreted into the glomerular filtrate by the proximal tubules [6]. Urea and creatinine levels rise above normal range in patients with metabolic syndrome. The findings of this study will also provide baseline data for evaluation of serum urea and creatinine.

Potassium is an intracellular cationic electrolyte that is necessary for normal cellular function. Because it is easily excreted by the kidneys rather than stored in the body, humans need a constant supply of potassium [7]. Metabolic syndrome is associated with obesity, and in a recent study, it was observed that high potassium intake could not reduce the risk of obesity (pooled OR = 0.78; 95% CI: 0.61–1.01), while serum potassium and urinary sodium-to-potassium ratio was associated with obesity [8]. Potassium intake was associated with metabolic syndrome (pooled OR = 0.75; 95% CI: 0.50–0.97) [7]. Sodium and potassium are important electrolytes in the body [8]. It is important to analyse the ratio of sodium to potassium and use it to determine the renal effects of metabolic syndrome.

In Africa, herbal medicine has always been a thing for our forefathers even without having any scientific backing to it. Bitter leaf has been believed to cure many ailments and wounds. It is my duty as a scientist to discover if there is any scientific proof to this claim. Hence, the need for this research. However, in the course of this research, I'll be focusing on the effects of bitter leaf on metabolic syndrome, which is a combination of different ailments at once. The aim of this study is to evaluate the renoprotective effects of extracts of bitter leaf (*Vernonia amygdalina)* on animal models of metabolic syndrome in rats.

Materials and Methods

Plant Materials

The bitter leaf (*Vernonia Amygdalina*) used for the study was obtained from Ogbete market, a local market in Enugu, Enugu state, Nigeria. The plant material was authenticated by a consultant taxonomist at the herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Chemicals and Reagents

Sucrose, cholesterol, vanaspati ghee and coconut oil were purchased from Ogbete market in Enugu. Reagent(s) were from Randox Laboratory Ltd, UK.

High-fat, High-Sucrose Diets for Induction of Metabolic Syndrome

High fat was prepared by mixing vanaspati ghee and coconut oil in the ratio of 3:1 (v/v). It was given to the rats at a dose of 3 ml/kg body weight per day. High sugar diet consisted of 30% sucrose solution (SS) which was orally fed to the rats. In addition, a mixture 50g of commercially available cholesterol powder and 9g of sodium deoxycholate (bile salt added to increase bioavailability) was dissolved in coconut oil and made up with the same solvent to 200ml to give 250mg/ml of high cholesterol diet (HCD). It was given to the rats at a dose of 500mg/kg body weight per day.

Animals

Twenty (20) adult albino rats, weighing 91.0g – 139.7g, were obtained from the animal house of the College of Veterinary Medicine, University of Nigeria. The animals were housed in metallic under standard conditions of temperature (22 ± 3^{o}C) and a 12 h light, 12 h dark cycle. The animals were kept under observation for about 14 days before the onset of the experiment for acclimatization. Experimental protocol and handling were according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings [9].

Preparation of Plant Extracts

Fresh healthy (400g) of Bitter Leaf (*Vernonia Amygdalina*) were washed, cut into small pieces and homogenized in a warring blender. The resulting mixture was soaked in 2 L of 80% methanol. The mixture was allowed to stand for 24 h with intermittent shaking. Following filtration, the filtrate obtained was concentrated to dryness at 40°C using a rotary evaporator under reduced pressure. The dried methanol extracts of *Vernonia Amygdalina* (MEVA) were weighed and then stored in a refrigerator at 4^{o}C.

Phytochemical Analysis of the Bitter leaf

Preliminary phytochemical screening of the Bitter Leaf for the presence of glycosides, flavonoids, saponins, steroids,

tannins, carbohydrates, proteins and terpenoids was carried out at Department of Pharmacognosy, Faculty of Pharmaceutical Science, University of Nigeria Nsukka. Methods according to Trease and Evans [10] were used for the analyses.

Experimental Design

The twenty (20) albino rats were grouped into (A-D) of five rats each and the received the following treatments which lasted for 4 weeks:

- Group A: (Negative Control): received oral administration of Ghee (3 ml/kg) + HCD (500mg/kg) + SS (10ml/kg).
- Group B: received oral administration of Ghee (3 ml/kg) + HCD (500mg/kg) + SS (10ml/kg); and low dose of MEVA (200mg/kg, oral).
- Group C: received oral administration of Ghee (3 ml/kg) + HCD (500mg/kg) + SS (10ml/kg); and high dose of MEVA (400mg/kg, oral).
- Group D: (Normal Control): No treatment was administered to this group.

Sacrificing of Animals and Sample Collection

Blood samples for the determination of serum analyses of creatinine, urea, sodium and potassium were taken by cardiac puncture of the left ventricle of the heart under chloroform anesthesia and the kidney was harvested for histopathological analyses.

Ethical Approval

Experimental protocol and handling were according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings [9].

Biochemical Analysis

The serum obtained was used for the analyses of urea, creatinine, sodium and potassium.

Determination of serum electrolytes

Serum electrolytes were determined usingPerlong Medical PL1000A Electrolyte Analyser. The electrolyte analyser applies the principle of advanced ion-selective electrode, which gives the instrument a stable and reliable measurement. It measures the ion concentrations of K+, Na+, Cl-, Ca++, HCO3, and pH values in the whole blood, serum and urine sample.

Determination of Blood Urea Nitrogen

Serum urea concentration was determined using the diacetylmonoxime method with protein precipitation according to Natelson *et al* [11]. Calculation: Calculation of Blood Urea Nitrogen BUN = Urea / 2.14.

Determination of serum creatinine concentration

Serum creatinine concentration was determined using the Jaffe Reaction according toFabing and Ertingshausen [12].

Histopathological Analysis

The excised kidneys were processed using the paraffin wax embedding technique, sectioned at 5 microns and stained using the Haematoxylin and Eosin [H and E] staining procedure [13]. The histological sections were examined using an Olympus TM light microscope.

Statistical Analysis

Data analysis was done using GraphPad prism version 7.0 (GraphPad, San Diego, CA, USA). The results of the biochemical assays were reported as mean±SEM (standard error of mean). The level of significance was tested using one way analysis of variance (ANOVA), followed by the Tukey post hoc analysis. Probability levels less than 0.05 ($p<0.05$) was considered significant.

Results

Biochemical Results

Albino Wistar rats that received oral administration of Ghee (3 ml/kg) + HCD (500mg/kg) + SS (10ml/kg) alone (negative control) for 3 weeks showed a statistically significant ($P<0.05$) elevated levels of blood urea nitrogen (BUN), creatinine and potassium (K^+) when compared with high dose treatment group and the normal control, separately (Table1). This showed that the Ghee (3 ml/kg) + HCD (500mg/kg) + SS (10ml/kg) alone induced renopathy secondary to metabolic syndrome in the rats. The treatment with low dose of MEVA (200mg/kg, oral) or high dose of MEVA (400mg/kg, oral) was shown to induce a significant decrease in blood urea nitrogen (BUN), creatinine and potassium (K^+) levels and improved the condition compared with the negative control groups. (Table 1)

Histopathological Results

In Figure 4, The glomeruli appear normal while some tubules appear to have slightly degenerated epithelia. The kidney section of rats that received oral administration of Ghee (3 ml/kg) + HCD (500mg/kg) + SS (10ml/kg) alone (negative control) for 3 weeks showed some glomeruli appear mildly constricted or eroded while the tubules appear normal (Figure 1). However, in Ghee + HCD + SS+ MEVA (Low dose)-treated group, it was observed that the glomeruli appear normal while some tubules appear eroded with some having intraluminal eosinophilic (Figure 2). Furthermore, the glomeruli of Ghee + HCD + SS+ MEVA (High dose) group rats, casts the glomeruli appear normal while the tubules appear normal with some tubules having intraluminal eosinophilic casts (Figure 3). The histopathological findings were in tandem with the biochemical results.

Table 1: Comparison of serum renal biochemical parameters of treated groups with normal controls

Groups	BUN (mg/dl)	Creatinine (mg/dl)	K^+ (mmol/l)	Na^+ (mmol/l)
Ghee + HCD + SS	37.92 ± 4.21	1.62 ± 0.04	7.68 ± 0.09	135.41 ± 0.23
Ghee + HCD + SS+ MEVA (Low dose)	21.75 ± 3.48*	1.04± 0.05*	5.92 ± 0.24	140.02 ± 3.10
Ghee + HCD + SS+ MEVA (High dose)	21.68 ± 1.09*	0.92± 0.19*	6.19 ± 0.76*	143.88 ± 0.35*
Normal Control	20.62 ± 1.31*	0.89 ± 0.30*	5.83 ± 0.12*	145.25 ± 1.42*

Values given as Mean ± SEM. *$p<0.05$ is significant when Ghee + HCD + SS (negative control) is compared with all other groups.

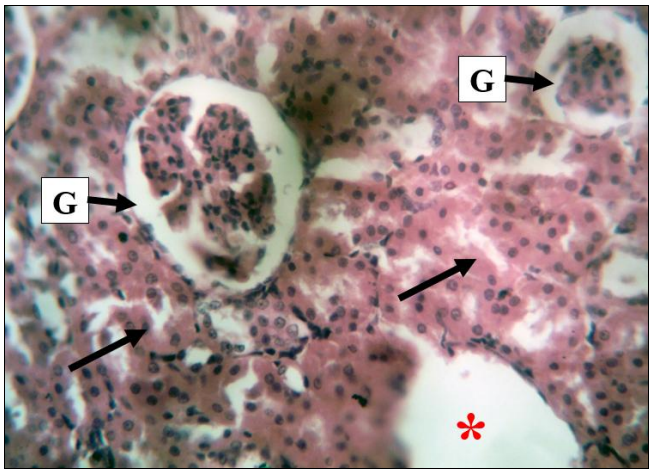

Fig 1: Representative micrograph of kidney of animals in group A. Some glomeruli (G) appear mildly constricted or eroded (*) while the tubules (arrows) appear normal. Stain: Haematoxylin and Eosin. Magnification: X400

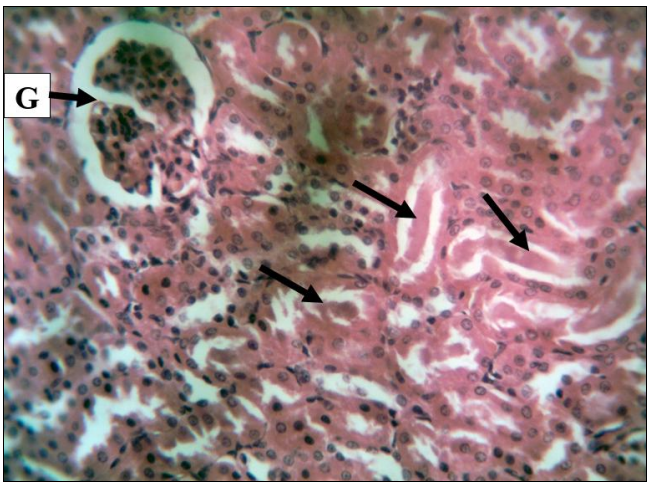

Fig 2: Representative micrograph of kidney of animals in group B. The glomeruli (G) appear normal while the tubules (arrows) appear normal with some tubules having intraluminal eosinophilic casts. Stain: Haematoxylin and Eosin. Magnification: X400

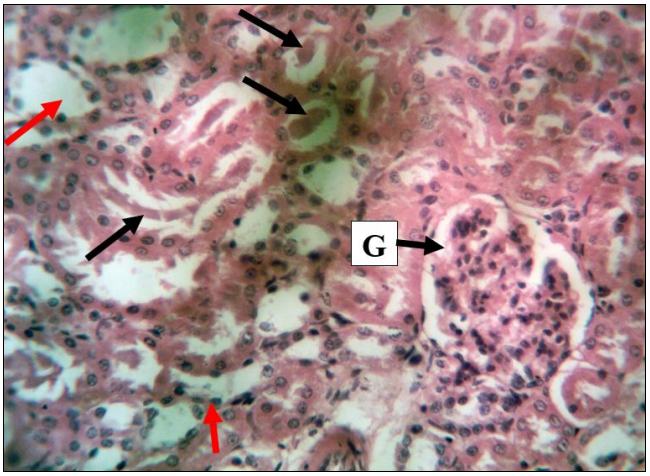

Fig 3: Representative micrograph of kidney of animals in group C. The glomeruli (G) appear normal while some tubules (arrows) appear eroded with some having intraluminal eosinophilic casts. Stain: Haematoxylin and Eosin. Magnification: X400

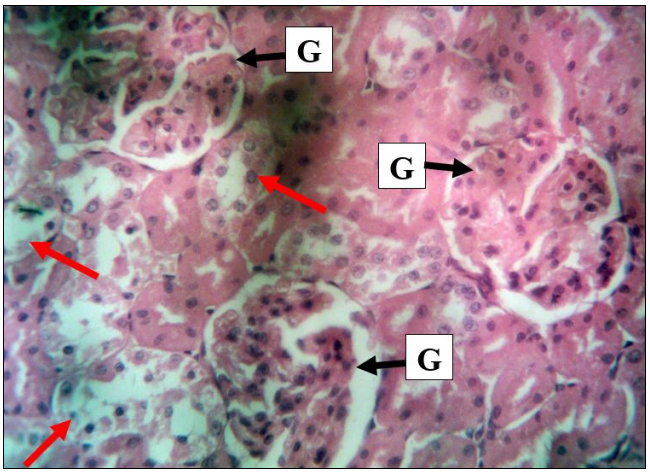

Fig 4: Representative micrograph of kidney of animals in group D. The glomeruli (G) appear normal while some tubules (arrows) appear to have degenerated epithelia. Stain: Haematoxylin and Eosin. Magnification: X400

Discussion

Excessive intake of certain food substances such as fatty foods, high cholesterol diets, sugary food and drinks leads to a combination of illnesses such as central obesity and abdominal obesity, systemic hypertension, insulin resistance (or type 2 diabetes mellitus), atherogenic dyslipidaemia. This combination is known as metabolic syndrome [1].

Metabolic syndrome affects different organs in the body, such as kidney, heart, liver, etc, but for the sake of this research, we have focused on the effects of metabolic syndrome on the kidney. In this study, the biochemical tests carried out to test for the functionality of the kidneys include; urea, creatinine, sodium and potassium. Histopathological tests were also carried out on the kidney biopsies.

The negative control groups for 3 weeks showed a statistically significant ($P<0.05$) elevated levels of blood urea nitrogen (BUN), creatinine and potassium (K^+) when compared with high dose treatment group and the normal control, separately. Moreso, the histopathological analysis of said negative control group showed some glomeruli appearing slightly constricted or eroded, while the tubules appear normal, whereas, the glomeruli of the high and low dose MEVA-treated groups as well as the normal control group appeared normal with some tubules having intraluminal eosinophilic casts. Thus, it could be said that administration of Methyl Extract of Vernonia Amygdalina (MEVA) has a restorative effect on kidney function in metabolic syndrome, as evidenced in the biochemical and histopathological analysis.

Current therapeutic options for metabolic syndrome in individuals are limited to treatment for hypertension, hyperglycaemia, and hypertriglyceridemia, as well as dietary control measures and regular exercise [1]. However, traditional alternatives such as African bitter leaf (Vernonia amygdalina) has been acclaimed to have a healing effect on the different conditions pertaining to metabolic syndrome (obesity, hypertension, insulin resistance, and dyslipidaemia). One study found a 21.4% decrease in blood glucose levels of diabetic rats that were administered with

bitter leaf extract after 14 days [14]. Another animal study discovered that bitter leaves significantly lowered fasting blood sugar, total cholesterol, triglyceride, and LDL-Cholesterol after fourteen days of administration [15].

In this study, the treatment with low dose of MEVA (Methanol Extract of Vernonia amygdalina) (200mg/kg) or high dose of MEVA (Methyl Extract of Vernonia amygdalina) (400mg/kg) was shown to induce a significant decrease in blood urea nitrogen (BUN), creatinine and potassium (K^+) levels and improved the condition compared with the negative control groups. Furthermore, we observed that the extracts had a dose-dependent protection, with the high dose (400mg/kg) showing better protection than the low dose (200mg/kg). In MEVA low dose-treated group, it was observed that the glomeruli appear normal while some tubules appear eroded with some having intraluminal eosinophilic, whereas in MEVA high dose-treated group, casts the glomeruli appear normal while the tubules appear normal with some tubules having intraluminal eosinophilic casts. Hence, the histopathological findings were in tandem with the biochemical results.

Conclusion

The findings from this study showed that the extracts of bitter leaf (*Vernonia amygdalina*) when given in high dose (400mg/kg) ameliorated the effects in the test group. Thus, the results suggests that sufficient dose of methanolic extracts of Vernonia amygdalina (MEVA) has a renoprotective effect on metabolic syndrome.

References

1. McCracken E, Monaghan M, Sreenivasan S. Pathophysiology of the metabolic syndrome. Clinics in Dermatology. 2018; 36(1):14-20.
2. Obidike J. Bitter leaf (Vernonia amygdalina): Benefits, uses, and side effects. Healthful Wonders, 2021. https://www.healthfulwonders.com/bitter-leaf-vernonia-amygdalina-benefits-uses-side-effects/
3. Uchendu IK. Effect of aqueous extract of bitter leaf (Vernonia amygdalina) against acetaminophen-induced liver damage in rats. Bioequivalence and Bioavailability International Journal. 2018; 2(1):000122.
4. Kawamoto R, Akase T, Ninomiya D, Kumagi T, Kikuchi A. Metabolic Syndrome is a Predictor of Decreased Renal Function Among Community Dwelling Middle-Aged and Elderly Japanese. Int Urol Nephrol. 2019; 12:2285-2294.
5. Higgins C. Urea and the clinical value of measuring blood urea concentration. Acutecaretesting. Org. 2016; 22:1-6.
6. Kene K, Wondimnew T, Welde M, Mateos T, Adugna T, Gerema U, *et al*. Prevalence and determinants of Impaired Serum Creatinine and Urea among type 2 diabetic patients of jimma medical center, Jimma, Southwestern Ethiopia, 2019. Endocrine and Metabolic Science. 2021; 3:100096.
7. Cai X, Li X, Fan W, Yu W, Wang S, Li Z, *et al*. Potassium and obesity/metabolic syndrome: A systematic review and meta-analysis of the epidemiological evidence. Nutrients. 2016; 8(4):183.
8. Li X, Guo B, Jin D, Wang Y, Jiang Y, Zhu B, *et al*. Association of dietary sodium: Potassium ratio with the metabolic syndrome in Chinese adults. British Journal of Nutrition. 2018; 120(6):612-618.
9. American Diabetes Association. Clinical practice recommendations, Diabetes Care. 2005; 28:S1-79.
10. Trease G, Evans SM. Pharmacognosy; 15th Edition, English Language Book Society BaillereTindall, London, 2002, 23-67.
11. Natelson S, Scott ML, Beffa C. A rapid method for the estimation of urea in biologic fluids. Am J ClinPathol. 1951; 21(3):275-281.
12. Fabing DL, Ertingshausen G. Automated reaction-rate method for determination of creatinine with the centrifichem. J. Clin. Chem. 1971; (17):696-700.
13. Baker FJ, Silverton RE, Pallister CJ. B S ' I L Technology. 7th Ed., Butterworth-Heinemann, Woburn, MA., USA., ISBN-13: 978075621908; 1998, 448.
14. Atangwho IJ, Egbung GE, Ahmad M, Yam MF, Asmawi MZ. Antioxidant versus anti-diabetic properties of leaves from Vernonia amygdalina Del. growing in Malaysia. Food Chemistry. 2013; 141(4):3428-3434.
15. Ogbuagu EO, Airaodion AI, Ogbuagu U, Airaodion EO. Effect of methanolic extract of Vernonia amygdalina leaves on glycemic and lipidaemic indexes of Wistar rats. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2019; 7(3):1-4.