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# Phytochemical Analysis and Anti-Hyperlipidemic Potential of Ethanolic Extract of *Hunteriaumbellata* Seed

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***Abstract:***
***Background:*** *Hyperlipidemia characterized by the abnormal elevation of plasma lipoprotein is the most prevalent indicator for cardiovascular diseases. This study screened, analyzed and accessed the anti-hyperlipidemic potentials of the phytochemicals and anti-nutrient compositions of ethanolic extracts of Hunteriaumbellata seeds with a view to utilizing the plants in the management of obesity and hyperlipidemia, and early stage renal dysfunction using high fat diet model in rats.*
***Materials and Methods****: Thirty healthy albino rats were randomly divided into five equal groups: Group I received normal saline (2 ml/kg bwt); Group II received a high fat diet; Group III received Orlistat (50 mg/kg bwt);Group IV Orlistat (50 mg/kg bwt) + H. umbellata (200 mg/kg bwt); Group V received Orlistat (50 mg/kg bwt) + H. umbellata (400 mg/kg bwt); The hepatocellular injury markers (aspartate transaminase (AST) and alanine transaminase (ALT)), kidney injury markers (urea and creatinine ) and lipid profile (total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) were estimated using standard Randox diagnostic kits, Low Density Lipoprotein (LDL) concentration were calculated using Friedewald's equation*
***Results****: The results revealed that H. umbellata seed has nutrients and anti-nutrients within permissible limits. Also revealed that HFD increased the body weight and all the assayed biochemical parameters, post-treatment with 400 mg/kg H. umbellata seed significantly ($p < 0.05$) decreased the concentrations of TG, cholesterol and LDL, Also post-treatment with 200 mg/kg plant extactsignificantly ($p < 0.05$) decreased urea and creatinine concentrations when compared to the HFD group. The significant decrease was quite better than the Orlistat treated group.*
***Conclusion:****These results revealed that the ethanolic extract of H.umbellataseed possesses safe to consume anti-nutrients and phytochemicals which elicited anti-hyperlipidemic potentials and normalizes the kidney injury markers at 400 and 200 mg/kg body weight respectively*
***Key Word****: Hunteriaumbellata; Hepatocellular injury; Kidney injury; Hyperlipidemic; Phytochemicals*

## I. Introduction

Abnormal and excessive fataccumulation isone of the major risk factors for a number of metabolic disorders like cardiovascular diseases, diabetes and cancer. Once considered high-income countries' problem, obesity and overweight is fast becoming a problem in urban areas of low-income countries mainly as a consequence of lifestyle changes[1]. Pre-clinical studies have shown a direct correlation between a high fat diet and overweight/obesity among distinct animal models[2]. An important aspect not often discussed is the relationship between the overweight and obesity caused byanhypercaloric and high fat diet and the development of kidney failure. Excess weight gain and obesity may cause renal changes, including glomerular hyperfiltration, sodium retention, enlargement of Bowman's space, increased glomerular cell proliferation, mesangial matrix expansion, inflammatory cell infiltration and tubulointerstitial lesions[3]. These early renal alterations can progress to more intense and diffuse lesions in the kidneys, such as focal segmental glomerulosclerosis, proteinuria and tubule-interstitial lesions, which are observed in prolonged obesity[4].

In addition, it hasbeen described that changes in lipoprotein metabolism and serum lipids occur with increasing renal dysfunction[5]. Moreover, the high levels of cholesterol andobesity have been associated with changes of glomerular structure during the final stages of renal disease[6].

There is therefore the need to arrest the trend using relatively assessed plant-based drug with little or no side effects.*H. umbellata*which belongs to the family of apocynaceae, is a plant native to West Africa and

reputed to be effective in the treatment and management of diverse ailments ranging from diabetes mellitus, gastric ulcers, liver disease, infections, hypertension and obesity. The plant has been reported to contain tannins, alkaloids, cardiac glycosides, saponins, flavonoids and anthraquinone, and possess antioxidant, anti-inflammatoryand aphrodisiac properties[7,8]. Adeneye*et al.*,reported the effectiveness of *H. umbellata* in the management of obesity andhyperlipidemia in rats induced with triton and olive oil.

This study is designed to evaluate the effect of *H. umbellata*seed extract in rats fed with high fat diet with a view to ascertaining its effectiveness in the management of obesity and hyperlipidemia, and early stage, renal dysfunction using the high fat diet model in rats.

## II. Material And Methods

A total 30 adult male Albino rats (both male and females) that weighed 120 – 130 g wereobtained from the Animal House, Faculty of Pharmacy, ObafemiAwolowo University, Ile-Ife. The rats werehoused in the Animal House, Department of Biochemistry, Adeleke University,Ede, Osun stateand kept under standard conditions.The rats were acclimatized for 14 days; fed with standard chow and water *ad libitum.*

**Study Design:***Invitro* and *invivo* study
**Plant Materials**: Dried seeds of *Hunteriaumbellata*were purchased from Ipetu-modu, Ife North Local Government, Osun state, Nigeria.The seeds were pulverized using a mechanical blender.

**Preparation of Ethanolic Extract***:* The ethanolic extract of *Hunteriaumbellata*was prepared according to a modified method described by16 Donald *et al.*, 2016. Typically, 500 g of pulverized plant material was defatted with n-Hexane (2000 ml) for 24 hours and was extracted with ethanol (1500 ml) for 48 hours. The suspension was stirred and filtered through a muslin cloth and Whatman No 1 filter paper. The residue was re-extracted five more times with ethanol until the filtrate was clear and became colourless. The filtrates were combined, allowed to settle, carefully decanted and re-filtered using Whatmann's No 1 filter paper. The combined filtrate was concentrated to dryness using a rotary evaporator. The extract was kept in the refrigerator for biochemical studies.

**Study Duration:**
**Subjects &Treatment method**: Thirty (3 male Albino rats was purchased and selected randomly for the purpose of this study. The animals were randomly allocated and orally gavagedas follows:
Group A(N = 6 rats)– standarddiet withdistilled water (Control)
Group B (N = 6 rats) – High Fat Diet only
Group C(N = 6 rats)– High Fat Diet + 50 mg orlistat/kg bwt
Group D(N = 6 rats) – High Fat Diet + 200 mg extract/kg bwt
Group E(N = 6 rats) – High Fat Diet + 400 mg extract/kg bwt

**Sacrificing and Samples Collection:** The animals were sacrificed under mild anasthesia with diethyl ether, twenty four hours after the last treatment (oral administration of extract and orlistat). Blood was collected via cardiac puncture into bottles containing anticoagulant (trisodium citrate, 3.8% w/v) and mixed gently.Blood sample was centrifuged on Bench Centrifuge Model 90-2 (Searchtech Instrument England, UK.) at3000 rpm for 10 min. The plasma was carefully transferred into clean vial bottles and kept frozen at -4°C for further biochemical assays.Liver (1g) and kidney (1 g) was cut into bits and homogenized in a total of 10 ml with freshly prepared phosphate buffer (pH 6.8, 100 mM). The homogenate was centrifuged at 3000 rpm with Bench Centrifuge at 3000 rpm for 10 min. The supernatant was carefully transferred into clean vial bottles and kept frozen at -4°C for further biochemical assays.

**Procedure methodology**

The proximate analysis, mineral composition[9] and phytochemical screening[10,11] of the ethanolic extract of *Hunteriaumbellata*seeds was carried out. The quantification of tannin[13], saponin[14] and phytate[15] in the seed ethanolic extract was also determined.

All lipid parameters were quantified on the blood plasma; Total cholesterol, High density lipoprotein-Cholesterol (HDL-c) and Triglyceride quantization was determined using standard kits from Randox Diagnostics®. Low density lipoprotein-Cholesteol (LDL-C) and very low density lipoprotein-Cholesterol (vLDL-C) was calculated using the Friedewald'sequation[18].

The hepatocellular markers; Aspartate transaminase (AST) and Alanine transaminase (ALT), and kidney biomarkers (urea and creatinine) were determined using standard kits from Randox diagnostics®.

Information about the type of orlistat was taken from the pharmacy database where it was purchased.

Body weights of the rats were measured once a weekof the experiment, using digital mettler (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The weight difference to the initial weight per group was calculated

**Statistical analysis**
Datawere expressed as mean ± SEM. Differences between the mean values of the control and treatment groups were determined by One-way Analysis of Variance withDunnettpost hoc test using the Graph Pad Prism 5. Significant difference was considered if $p < 0.05$.

## III. Result

In Table no 1is the result of thephytochemical screening of the ethannolic extract of *Hunteeriaumbellata* seed. Itrevealed that cardio glycoside was strongly positive, followed by flavonoids, terpenoids and saponin, that are moderately positive. While the tannins and steroids were slightly positive.

**Table no 1:** Photochemical screening of ethanolic extract of *Hunteriaumbellata.*

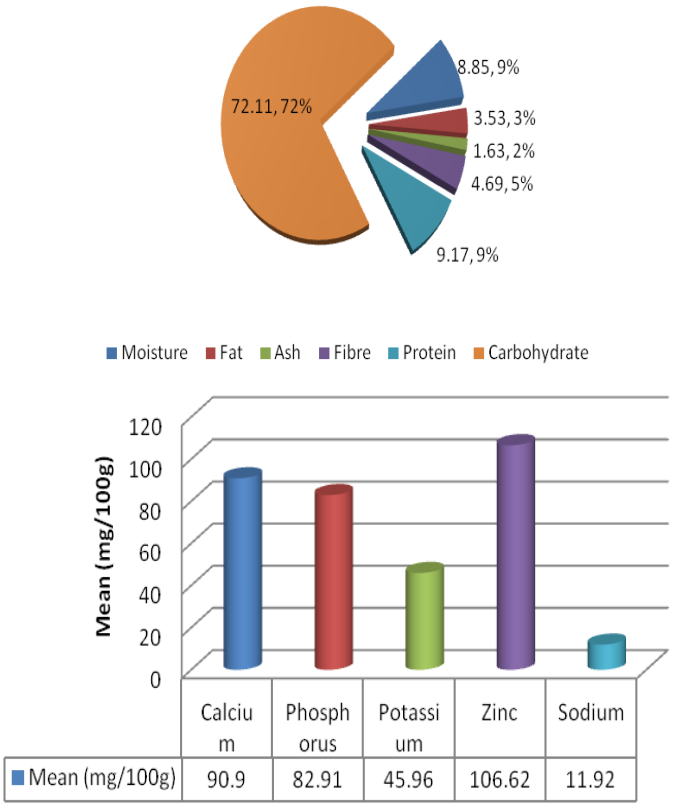
| Phytochemicals | Status |
|---|---|
| Tannins | + |
| Saponin | ++ |
| Steroids | + |
| Terpenoid | ++ |
| Flavonoids | ++ |
| Cardiac glycoside | +++ |

**Proximate Analysis, Mineral and anti-nutrient Composition of *Hunteriaumbellata***
The Proximate Analysis and Mineral Composition of the *Hunteriaumbellata* indicated that the seed contain 72.11% of carbohydrate which is the highest followed by protein, moisture, fiber and fat. Ash is the least present.
The mineral composition was also estimated as mg per 100 g of the seed. The result indicated zinc is the most abundant, followed by calcium, phosphorus, potassium, and with sodium as the least present mineral.

In Table no 2 is the result of the proximate analysis and mineral compositionof the seed of *Hunteriaumbellata.* The carbohydrate, 72.11± 0.80 %, Protein, 9.17 ± 0.08 %, Moisture, 8.85 ± 0.06 %, Fiber, 4.69 ± 0.35 %, Fat, 3.53 ± 0.44% and Ash 1.63 ± 0.02%. The minerals include; Zinc 106.62 ± 0.14 mg/100g, Calcium 90.9 ± 0.08 mg/100g, Phosphorus 82.91 ± 0.01 mg/100g, Potassium 45.96 ± 0.02 mg/100g and Sodium 11.92 ± 0.14 mg/100g

**Table no 2:**Proximate analysis and mineral composition of *Hunteriaumbellata seed*

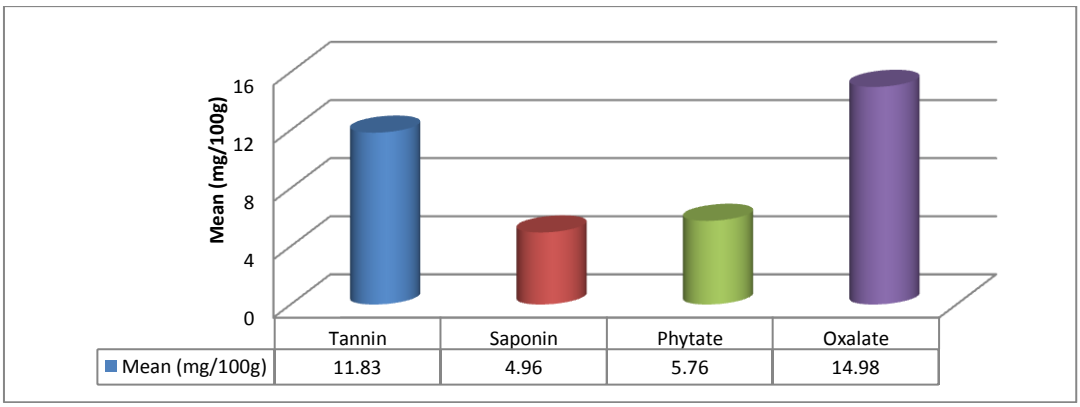
| Proximate (%) | | Mineral(mg/100g) | |
|---|---|---|---|
| Moisture | 8.85 ± 0.06 | Calcium | 90.9 ± 0.08 |
| Fat | 3.53 ± 0.44 | Phosphorus | 82.91 ± 0.01 |
| Ash | 1.63 ± 0.02 | Potassium | 45.96 ± 0.02 |
| Fiber | 4.69 ± 0.35 | Zinc | 106. 62 ± 0.05 |
| Protein | 9.17 ± 0.08 | Sodium | 11.92 ± 0.14 |
| Carbohydrate | 72.11 ± 0.80 | | |




In Table no 3 is the anti-nutrient composition of ethanolic seed extract of *Hunteriaumbellata.* Oxalate (16.98 ± 0.44 mg/100g) is the most abundant anti-nutrient, with levels above the permissible limit. Phytate (5.67 ± 0.11 mg/100g), saponin (4.96 ± 0.07 mg/100g) and tannin (11.83 ± 0.05 mg/100g) are well below the permissible limit.

**Table no3:** Anti-nutrients composition of Ethanolic seed extract of *Hunteriaumbellata*

| | *H.umbellata* (mg/100g) | Permissible limit (mg/100g) (Ndidi*et al.*, 2014) |
|---|---|---|
| Tannin | 11.83 ± 0.05 | 2000 |
| Saponin | 4.96 ± 0.07 | 50 – 200 |
| Phytate | 5.76 ± 0.11 | 250 – 500 |
| Oxalate | 14.98 ±0.44 | 0.3 – 0.5 |




**Effect of *H. umbellata* on Body Weight Gain**

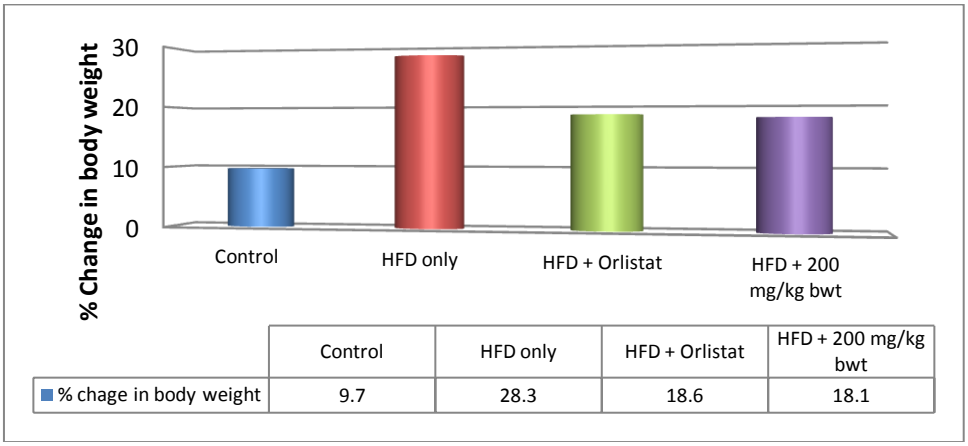
In Table no4is the result of Percentage (%)Change in body weight (g) on the ethanolic extract of *Hunteria umbellate*. Therats fed with HFD showed the highest percentage change in body weight. The percentage change

in body weight of rats fed with 200 mg/kg bwt and 400 mg/kg bwtwas reduced even better than the standard Orlistat drug.

The percentage change in weight of the the animals were as follows: control group: 9.7 %, the HFD only: 28.3 %, HFD + Orlistat: 18.6 %, HFD + 200 mg/kg bwt: 18.1 % and HFD + 400 mg/kg bwt, 15.4%.

**Table no4:** Percentage (%) Change in body weight (g) on the ethanolic extract of *Hunteriaumbellata*

| Groups | Initial weight (g) | Final weight (g) | Percentage Change | P value |
|---|---|---|---|---|
| Control | 139.95 ± 5.58 | 153.60 ± 3.79 | + 9.7 | <0.01 |
| HFD only | 129.70 ± 7.38 | 166.40 ± 6.53 | + 28.3 | <0.01 |
| HFD + Orlistat | 132.5 ± 7.94 | 157.2 ± 8.92* | + 18.60 | <0.01 |
| HFD + 200 mg/kg bwt | 128.00 ± 3.46 | 151.20 ± 4.35* | + 18.1 | <0.01 |
| HFD + 400 mg/kg bwt | 136.90 ± 8.42 | 157.90 ± 8.66* | + 15.4 | <0.01 |

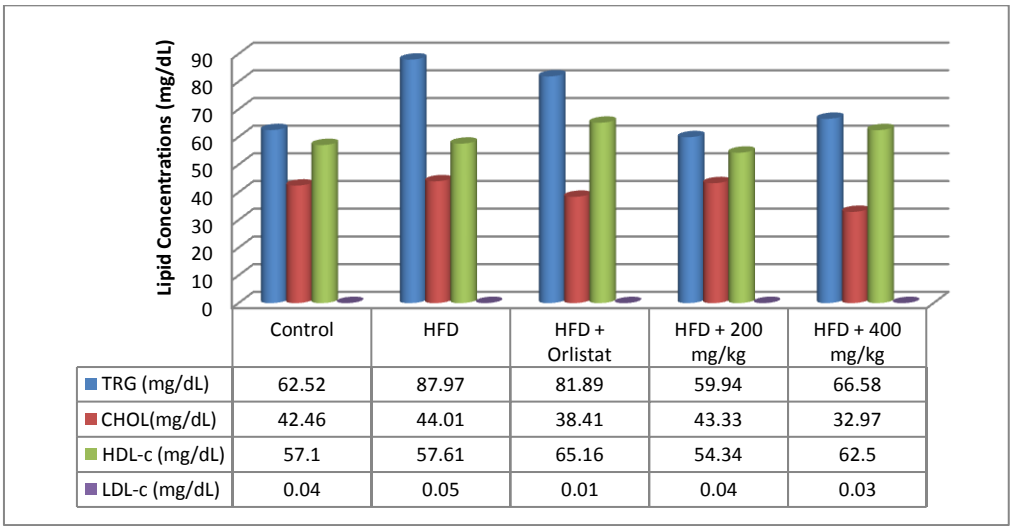



**Effect of *H. umbellata* on Lipid profile:**

In Table no 5is the resultof the effect of ethanolic seed extract of *H. umbellata* on plasma Lipid profile.*H. umbellata*at 400 mg/kg bwtsignificantly ($p < 0.05$) reduced the plasma triglyceride, cholesterol and LDL-c (66.58 ±1.03 mg/dl, 32.97 ±0.21 mg/dl, 0.03 ±0.01 mg/dl) as compared to the HFD group87.97 ±2.75 mg/dl, 44.01 ±0.43mg/dl, 0.05 ± 0.00mg/dl respectively), even better than the standard drug (orlistat group). Also, the plant extract at 400 mg/kg bwt significantly ($p<0.05$) increased the HDL-c (62.50 ±4.05 mg/dl) as compared to the HFD group (57.61 ±2.86 mg/dl).

**Table no5:**Effect of ethanolic seed extract of *H. umbellata* on plasma Lipid profile

| Groups | TRG (mg/dL) | CHOL(mg/dL) | HDL-c (mg/dL) | LDL-c (mg/dL) |
|---|---|---|---|---|
| Control | 62.52 ±0.87 | 42 46 ±0.48 | 57.10 ±4.22 | 0.04 ± 0.01 |
| HFD | 87.97 ±2.75 | 44.01 ±0.43 | 57.61 ±2.86 | 0.05 ± 0.00 |
| HFD + Orlistat | 81.89 ±0.76 | 38.41 ±0.46* | 65.16 ±4.43* | 0.01 ± 0.01* |
| HFD + 200 mg/kg bwt | 59.94 ±2.35* | 43.33 ±0.51 | 54.34 ±3.43* | 0.04 ± 0.01 |
| HFD + 400 mg/kg bwt | 66.58 ±1.03* | 32.97 ±0.21* | 62.50 ±4.05* | 0.03 ± 0.01* |
| p-value | p < 0.05 | p < 0.05 | p < 0.05 | p < 0.05 |

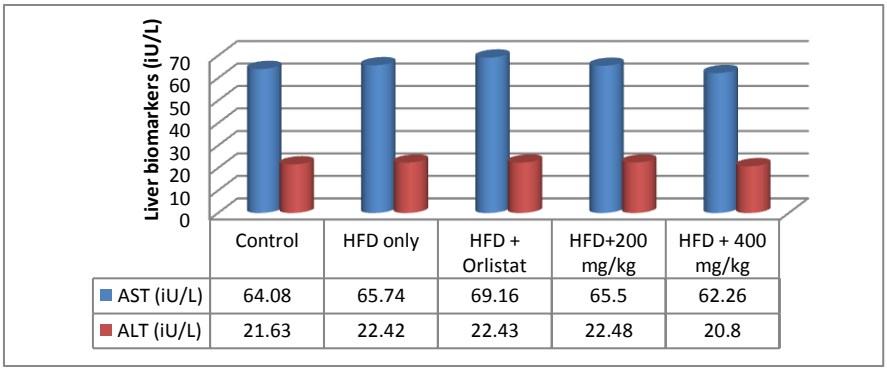


**Effect of *H umbellata* on Hepatocellular injury**

In Table no 6 is the resultof the effect of ethanolic extract of *H. umbellata*on hepatocellular injury. *H. umbellata*at 400 mg/kg bwt insignificantly ($p > 0.05$) reduced the AST and ALT (62.26 ± 0.76iU/L and20.80 ± 0.42iU/L) as compared to the HFD group (65.74 ± 1.95iU/L and 22.42 ± 0.68iU/L respectively), even better than the standard drug (orlistat group).

**Table no 6:** Shows the effect of ethanolic extract of *H. umbellata*on hepatocellular injury

| Groups | AST (iU/L) | ALT (iU/L) |
|---|---|---|
| Control | 64.08 ± 1.50 | 21.63 ±0.46 |
| HFD only | 65.74 ± 1.95 | 22.42 ± 0.68 |
| HFD + Orlistat | 69.16 ± 1.38 | 22.43 ± 0.75 |
| HFD+200mg/kg bwt | 65.50 ± 3.00 | 22.48 ± 0.61 |
| HFD + 400 mg/kg bwt | 62.26 ± 0.76 | 20.80 ± 0.42 |
| p-value | p > 0.05 | p > 0.05 |

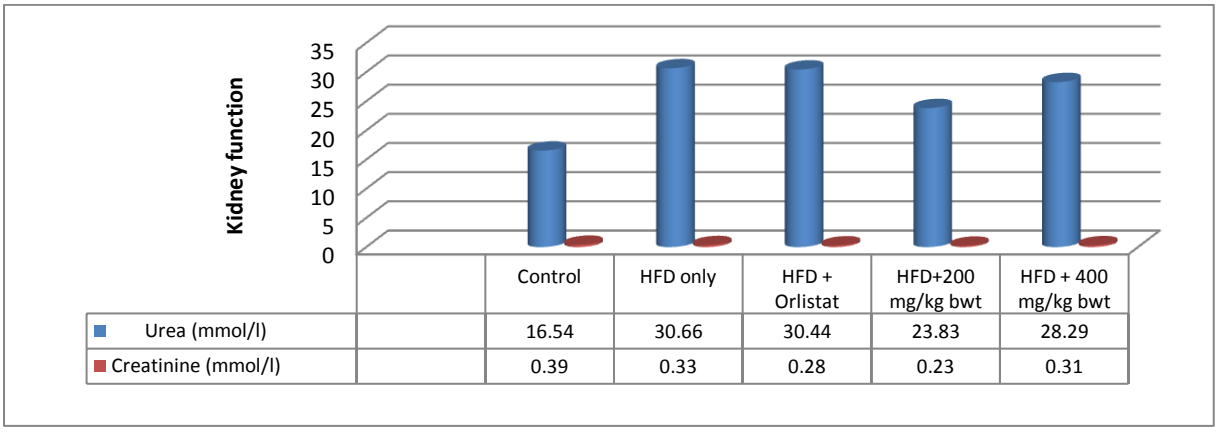



**Effect of *H umbellata* on Kidney function**

In Table no 6 is the effect of ethanolic extract of *H. umbellata*on kidney function. *H. umbellata*at 200 mg/kg bwt significantly ($p < 0.05$) reduced the Urea and Creatinine (23.83 ± 2.71mmol/L and 0.23 ± 0.03 mmol/L) as compared to the HFD group (30.66 ± 2.08mmol/L and 0.33 ± 0.03mmol/L respectively), even better than the standard drug (orlistat group).

**Table no 6:** Shows the effect of ethanolic extract of *H. umbellata*on Kidney function

| Groups | Urea(mmol/l) | Creatinine(mmol/l) |
|---|---|---|
| Control | 16.54 ± 2.08 | 0.39 ± 0.02 |
| HFD only | 30.66 ± 2.08 | 0.33 ± 0.03 |
| HFD + Orlistat | 30.44 ± 3.29 | 0.28 ± 0.02 |
| HFD+200mg/kg bwt | 23.83 ± 2.71* | 0.23 ± 0.03* |
| HFD + 400 mg/kg bwt | 28.29 ± 3.98* | 0.31 ± 0.02 |



## IV. Discussion

Phytochemicals are chemical compounds formed during plants normal metabolic processes; these chemicals are often referred to as secondary metabolites. The results of the present study revealed the presence of tannins, saponins, steroids, terpenoids, flavonoids and cardiac glycosides. Methanol and ethanol have been proved as effective solvents to extract phenolic compounds[20] which are found to be apparent in the ethanolicseed extract of *H umbellata*(Table 2). Cardiac glycosidewhich was stronglyindicated inethanolic extract of *H. umbellata*seedis a class of natural products able to increase the cardiac contractile force in patients with congestive heart failure and cardiac arrhythmias[21]. Flavonoids and Saponins have been established to possess antioxidant properties, reactive oxygen species scavenging potentials, and cell function modulation. These properties account for the large part of their pharmacological activity[22].

Nutritional and proximate analyses of plants are used to evaluate their nutritional importance as they are used as food by animals as well as being employed by humans for medicinal purposes[23]. The importanceof mineral elements in health maintenance and protectionagainst various diseases is well documented. The result of this study revealed that *H. umbellata* contained an appreciable amount of calcium, phosphorus, zinc and sodium. Zinc serves as biocatalyst,functions as a co-factor of many enzymes in the synthesis ofbiomolecules. Potassium and sodiumplay a key role in the maintenance of electrical potential, healthy centralnervous system; strong teeth and bone formation. Phosphorus helps in development of reproductive and immune systemsamong other functions[24].Calcium is essential for bone and teeth formation and development, blood clotting and for normal functioning of heart, nervous system and muscles. Calcium deficiency can lead to ricket, osteomalacia and tooth decay[25]. The seed of *H. umbellata* is a good source of carbohydrate for energy and a relative source of mineral (ash), protein and fat. The relatively low moisture content of *H. umbellate*indicates a possible stable plant shelf life[26].

In this present study, *H. umbellata*anti-nutrient contents (Tannin, Oxalate, Phytate and Saponin) were all safely below the permissible limits.Saponins have been shown to lower cholesterol concentration by competing against it for absorption in the body[25]. Dietary oxalate has been known to complex with calcium, magnesium, and iron leading to the formation of insoluble oxalate salts resulting in oxalate stones and interferes with utilization of the minerals. Phytic acid interferes with the bioavailability of nutrients in diet and inhibits several proteolytic enzymes and amylases[20]. Phytic acid is one of few chelating therapies for uranium removal. Phytic acid has some mineral binding properties. It may prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract[25,26]. Tannins bind to proline-rich proteins and interfere with protein synthesis and enzyme function[26]. Obesity, characterized by increased adipose tissue results from both increased fat cell number and increased fat size, develops from excessive energy intake and reduced energy expenditure[6].

In the present study, the rats fed HFD for three weeks showed overweight as indicatedby the significant increase in weight (Table 5). This is closely related to the significantly development of dyslipidemia. After treatment with *H. umbellata* extract, the body weight of the animals was significantly decreased. The result suggested that treatment of *H. umbellata*might have anti-hyperlipidemiceffect in HFD-induced obese rats.Ibeh*et al.*, reported that the exposure to extracts of abeere seed did not change significantly the body weights of affected rabbit, which suggests no adverse effect on metabolic activities of the rabbits treated with the seed extract[27].

Lipids are found in the blood and are stored in tissues; they play an important role in the body. Lipid disorders, such as high cholesterol, may lead to life-threatening illnesses, such as coronary artery disease (CAD), heart attack, or stroke[6].Lipid profile describes the level/status of different lipids: triglycerides, totalcholesterol, high-densitylipoproteincholesterol and low-densitylipoproteincholesterol in the blood. It serves as a medical screening tool for abnormalities in lipids[6].In this present study, (Table 6)*H. umbellata*at 200mg/kg significantlyattenuated the effect of the HFD on the level of triglyceride and HDL-C when compared to the standard drug (orlistat). The *H. umbellata* at 400 mg/kg significantly decreased the concentrations of cholesterol and LDL-c levels when compared to the HFD only fed rats. This revealed the anti-hyperlipidemic effect of ethanolic extract of *H. umbellata.*Akpabio and Ikpe (2013) reported that phyticacidhas been implicated as having a significant effect on reducing plasma cholesterol and levels of triglyceride, this effect is thought to berelated to the ability of phytic acid to bind to zinc and thuslower the ratio of plasma zinc tocopper which is known to dispose humans to cardiovascular disease[26]. Uzoekwe andMohammed,reported that the hypocholesterolemic effect of saponins[25].

The liver plays a central role in the maintenance of metabolic homeostasis[28]. The membranes of liver cells can become permeable when damaged, allowing for escape of intracellular enzymes into the bloodstream. The major intracellular enzymes are alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[28]. In this present study, there was no significant difference in the activities of liver indices (AST and ALT) of HFD fed rats when compared to the treated group (Table 7). This indicated that the HFD does not have effect on the liver. This was probably due to short term duration of the study. Several studies have reported increase in

activities of AST and ALT.Ibeh*et al.*, reported that the effect of abeere seed extracts on selected enzymes showed an enhancement in the activities of aspartate transaminase (AST) and alanine transaminase (ALT)[27].

The function of the kidneys is to remove wastes and excess fluid from the blood. Ureaandcreatinine are good markers that indicate regular functioning of the kidney. Urea is a waste product that is created by protein metabolism and excreted in the urine. Creatinineis a waste product of muscle energy metabolism and is produced at a constant rate that is proportional to the muscle mass of an individual. The body does not recycle it, so the quantity filtered by the kidneys in a given amount of timeis excreted with the urine, making creatinine clearance a specific measure of the kidney function[4].In this present study, there was significant increase it the concentration of creatinine and urea in the HFD fed animals when compared to the control group (Table 8). However, there was significant reduction when treated with the extract at 200 mg/kg bwt.There were no significant changes when treated with the extract at 400 mg/kg bwt and orlistat at 50 mg/kg bwt. The results obtainedrevealed that consumption of HFD might play a role in the development of kidney impairment associated with obesity and *H. umbellata*extract exhibits attenuation in the impairment, indicated with the improved plasma urea and creatinine levels in HFD-induced obese rats.This is in constrast to the result of Hall*et al*., who reported an insignificant increase in the levels of creatinine and urea[4].

## V. Conclusion

In conclusion, the results of this study revealed that *Hunteriaumbellata* seed has secondary metabolites and anti-nutrients within permissible limitswhich normalized the lipid profile and renal injury markers ofhigh fat diet-induced obese rats. The seed could be a good source of bioactive substances useful in the treatment and management of dyslipidemia and related disorders.Moreover, the level of plant's secondary metabolites indicated that the *H. umbellata* may serve a medicinal and therapeutic function.

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