***Original Research Article***

# VARIATION IN BODY WEIGHT, LIPID PROFILE AND SELECTED REPRODUCTION HORMONES IN RATS GIVEN PSIDIUM GUAJAVA LEAVES FROM CRUDE OIL POLLUTED AND NON-CRUDE OIL POLLUTED AREAS


## ABSTRACT

Variation in body weight, lipid profile and selected reproduction hormones in rats given *Psidium guajava* leaf samples from crude oil polluted and non-crude oil polluted areas was evaluated. Thirty-six albino rats of Wistar strain weighing between 90-120 g were divided into three major groups of I-III, with each group having two subgroups designated "a" and "b". Each of the subgroup housed six rats and they were given different concentrations of the compounded feed of the leaf samples. Rat groups placed on *P.guajava* leaf sample from non-crude oil polluted area had significantly ($p<0.05$) increased weight when compared to rat groups placed on *P.guajava* leaf sample from crude oil polluted area. Triglyceride, cholesterol and low density lipoprotein cholesterol (LDL-C) increased significantly ($p<0.05$) in rat groups placed on *P. guajava* leaf sample from crude oil polluted area against rat groups placed on *P. guajava* leaf sample from non-crude oil polluted area. Atherogenic indices of rat groups placed on *P. guajava* leaf sample from crude oil polluted area showed increased risk to cardiovascular diseases when compared to rat groups placed on *P. guajava* leaf sample from non-crude oil polluted area. The evaluated reproductive hormones increased significantly ($p<0.05$) in rats placed on *P. guajava* leaf sample from crude oil polluted area against those groups placed on *P. guajava* leaf sample from non-crude oil polluted area. The constituents of *P. guajava* leaf sample from crude oil polluted area could be behind the observed risk while the increase in hormones could be linked to increased cholesterol in rats groups placed on the leaf sample from crude oil polluted area. There is to sensitise those in the act of herbalism to be aware of where they harvest the plants they use as raw materials. This study has shown the variation in body weight, lipid profile and selected reproduction hormones in rats given *P. guajava* leaves from crude oil polluted and non-crude oil polluted areas.



**Keywords:** Body weight, lipid profile, *Psidium guajava*, crude oil polluted, hormones.


## INTRODUCTION

Herbalism, the act of using plants to remedy disease conditions is as old as mankind on this planet Earth [1]. The act solely makes use of plant materials addressed as medicinal plants [2]. According to Sofowora [2], medicinal plant is one whose one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The substances that could be responsible for the potency of medicinal plants have been identified as phytochemicals and phytonutrients [2-9]. These substances are biologically active in nature, and are physiologically active against disease pathogens [10-18].

*Psidium guajava* commonly called guava, is among those plants with phytochemicals and phytonutrients, which are biologically and physiologically active against disease pathogens [19]. The plant has a confirmed potency against different disease conditions [20]. *P. guajava* belongs to the family *myrtaceae* [11-19]. Parts of *P. guajava* are use in the act of herbalism

for preparation of syrups and concoctions used against diseases in traditional medicine. Studies have shown the lipidaemic, liver protective, haemapoetic, anti-diarrheal, antihypertensive, antioxidant, antimicrobal, hypoglycemic and antimutagenic potency of *P. guajava* [21-22]. Due to the position occupy by *P.guajava* in the practice of herbalism, any of its parts is indiscriminately collected when needed without taking into consideration the nature of the area or site where the tree is found. Considerations should be taken against collecting medicinal plants found in polluted environments to avoid taking any poison that comes which such pollution into the body [23].

The Niger Delta area of Nigeria harbours polluted environment where medicinal plants used in the act of herbalism are found [23]. The area is known for crude oil production which Nigeria is associated with. The environmental degradation associated with crude oil and refined crude oil products are synonymous with this area. Okrika, a port town in Rivers State is one of such towns found in Niger Delta area of Nigeria, which is associated with environmental degradation of crude oil and refined crude oil products [23]. It is on record that medicinal plants found within Okrika are employed in the act of herbalism. Since Okrika town harbours crude oil degraded environment on which medicinal plants grow, there is need to consider the possible effect of a known medicinal plant from such degraded environment in the body.

This study looked into that area and comparatively established variation in body weight, lipid profile and selected reproduction hormones in rats given *P. guajava* leaves from crude oil polluted area such as Okrika and non-crude oil polluted areas.

## MATERIALS AND METHODS

### Collection and Identification of Plant Materials

The plant materials used in this study were collected from a crude oil polluted site in Okrika Rivers State, and a botanical garden (Non-crude oil polluted site) found in Owerri, Imo State, both in Nigeria. The plant materials were identified by Professor Ferdinand Nkem Mbagwu of Department of Plant Science and Biotechnology, Imo State, University Owerri, Nigeria as *P. guajava*. Their leaves were collected, air dried and crushed with pestle and mortar, then sieved to obtain the coarse powder, which was used to compound the feed used for further studies.

### Experimental Animals

Thirty-six albino rats of Wistar strains weighing between 90-120 g were purchased from the animal colony of Department of Biochemistry, Gregory University, Uturu, Nigeria. The rats were allowed to acclimatize in their new environment for five days before they were used for studies. They were separated into three major groups of I-III, with each group having two subgroups designated “a” and “b”. Each of the subgroup housed six rats. The rats were given compounded feed of *P. guajava* and rat feed. The rat feed was a brand of commercial grower freshly obtained from a feed dealer along Abayi road, Aba.

Treatment given to the rats are as follows

Group Ia: 5% of *P. guajava* (crude oil polluted area) + 95% normal feed + potable water.

Group Ib: 5% of *P. guajava* (non-crude oil polluted area) + 95% normal feed + potable water.

Group IIa: 25% of *P. guajava* (crude oil polluted area) + 75% normal feed + potable water.

Group IIb: 25% of *P. guajava* (non-crude oil polluted area) + 75% normal feed + potable water.

Group IIIa: 50% of *P. guajava* (crude oil polluted area) + 50% normal feed + potable water.

Group IIIb: 50% of *P. guajava* (non-crude oil polluted area) + 50% normal feed + potable water.

The treatments of experimental rats were in accordance to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals [24]. The treatment lasted for 28 days.

**Biochemical Studies**

Rats from the various groups were weighed and sacrificed while under chloroform anesthesia after the treatment period. Blood was collected by direct cardiac puncture into tubes for lipid and hormonal studies. The tubes were properly labeled for analysis[25] Aside very low density lipoproteins, VLDL-cholesterol, the assays were performed according to their manufacturers' instructions using diagnostic test kits for the lipid profile parameters purchased from BioSystems® (S.A. Costa Brava of Barcelona, Spain). VLDL-cholesterol concentration was estimated using the methods of Burnstein and Sammaille [26]. LDL-cholesterol/ HDL-cholesterol ratio was estimated using simple mathematical method as reported by Duru et al [27]. The atherogenic indices were calculated as follows Cardiac Risk Ratio (CRR) = TC / HDL-C [28]; Atherogenic Coefficient (AC) = (TC – HDL-C)/ HDL-C [22]. Atherogenic Index of Plasma (AIP) = log (TG / HDL-C) [29].The instruction found in the kit for luteinizing hormone was adhered to for its estimation. Serum testosterone assay was carry out using tube based enzyme immunoassay (EIA) method [30].

**Statistical Analysis**

Results were presented as mean and standard deviation of triplicate determinations using Tables. Significant difference was established using students t-tests between two subgroups "a" and "b" of a main group at $p<0.05$.

**RESULTS AND DISCUSSION**

**Table 1**: Change in weight of rats given *P. guajava* leaves from crude oil polluted and non-crude oil polluted areas.

| | Group I | | Group II | | Group III | |
|---|---|---|---|---|---|---|
| Parameters | Ia | Ib | IIa | IIb | IIIa | IIIb |
| Final weight (g) | 159.31±1.90 | 183.88±1.22 | 155.23±3.18 | 177.27±2.96 | 139.70±134 | 170.80±2.14 |
| Initial weight (g) | 109.13±3.11 | 108.98±1.67 | 109.10±1.40 | 108.97±1.60 | 109.03±1.30 | 108.90±1.65 |
| Weight change (g) | 50.18±2.70 | 74.90±0.19* | 46.13±1.91 | 68.30±2.54* | 30.67±2.80 | 61.90±1.50* |

Results are presented as mean and standard deviation of triplicate determinations. Values of "b" subgroup asterisked against those of "a" subgroup under a main group on the Table are statistically significant at $p<0.05$.

Body weight change for rats placed on leaves of *P. guajava* ranged from 108.90 to 109.10 g (Table 1). Rats placed on leaves of *P. guajava* from oil polluted site (Ia, IIa and IIIa) had significantly ($p<0.05$) reduced body weight when compared to rats placed on leaves of *P. guajava* from non-crude oil polluted site (Ib, IIb and IIIb). The reduction in weight could be attributed to the contents of leaves of *P. guajava* from oil polluted site.

Table 2: Lipid profile (mg/dl) of rats placed on leaves of *P. guajava* from crude oil polluted and non-crude oil polluted areas.

| | Group I | | Group II | | Group III | |
|---|---|---|---|---|---|---|
| Parameters | Ia | Ib | IIa | IIb | IIIa | IIIb |
| Triglyceride | 115.19±2.90 | 97.33±1.80* | 102.12±4.30 | 91.13±2.10* | 119.90±1.60 | 95.73±2.08* |
| Cholesterol | 97.45±1.06 | 83.90±2.81* | 94.05±0.57 | 79.32±1.13* | 93.37±0.80 | 75.78±1.40* |
| LDL-C | 35.31± 1.43 | 21.33±2.15* | 32.54±1.32 | 12.14±2.64* | 25.46±1.82 | 6.44±0.82* |
| HDL-C | 39.10±0.87 | 43.10±1.23* | 41.09±1.50 | 48.95±1.46* | 43.96±1.54 | 50.19±1.32* |
| Non-HDL-C | 58.35±2.63 | 40.80±1.94* | 52.96±0.87 | 30.37±1.98* | 49.41±1.22 | 25.59±1.50* |
| VLDL-C | 19.16±1.90 | 16.20±0.73* | 18.64±046 | 15.64±1.23* | 18.47±1.54 | 15.08±1.22* |

Results are presented as mean and standard deviation of triplicate determinations. Values of "b" subgroup asterisked against those of "a" subgroup under a main group on the Table are statistically significant at $p<0.05$.

*LDL-C= Low density lipoprotein cholesterol; HDL-C=High density lipoprotein cholesterol; Non-HDL-C= Non-High density lipoprotein cholesterol; and VLDL-C-Very low density lipoprotein cholesterol.*

Lipid profile as present in Table 2 shows that triglyceride ranged from 91.13 to 119.90 mg/dl; cholesterol ranged from 75.78 to 97.45 mg/dl; LDL-C ranged from 6.44 to 35.31 mg/dl; HDL-C ranged from 39.10 to 50.19 mg/dl; Non-HDL-C ranged from 25.59 to 58.35 mg/dl; and VLDL-C ranged from 15.08 to 19.16 mg/dl. Triglyceride and cholesterol are both needed for the maintenance of healthy cells in the body [31-33]. However, their high levels have been associated with coronary artery disease [32-33]. Higher risk of heart and blood vessel disease have been linked to high level of triglyceride [31-33]. Triglyceride ranged from 91.13 to 119.90 mg/dl [Table 2], and significantly increased ($p<0.05$) in rats placed on leaves of *P. guajava* from crude oil polluted site (Ia, IIa and IIIa) when compared to rats placed on leaves of *P. guajava* from non-crude oil polluted site (Ib, IIb and IIIb respectively). Rats in groups Ia, IIa and IIIa had significantly increased cholesterol ($p<0.05$) when compared to rats of Ib, IIb and IIIb groups respectively. LDL-C is regarded as bad cholesterol. High level of LDL-C has been linked to an increased risk of heart and blood vessel disease [26]. LDL-C increased significantly ($p<0.05$) in groups Ia, IIa, and IIIa against their respective Ib, IIb and IIIb in this study. The good cholesterol of the body is high density lipoprotein (HDL-Cholesterol) [33]. Levels of HDL-C reduced significantly ($p<0.05$) in rat groups (Ia, IIa, and IIIa) placed on leaves of *P. guajava* from crude oil polluted site when compared to respective rat groups (Ib, IIb and IIIb) placed on *P. guajava* from non-crude oil polluted site. This observation could imply that rats placed on leaves of *P. guajava* from crude oil polluted site may be exposed to increased risk of heart and blood vessel disease than those placed on leaves of *P. guajava*

from non-crude oil polluted site. It has been reported that non-HDL cholesterol (Non-HDL-C) is a better predictor of cardiovascular risk than LDL-C. Non-HDL-C levels of rats groups (Ia,IIa and IIIa) placed on leaves of *P. guajava* from crude oil polluted site increased significantly ($p<0.05$) when compared respectively to rats groups (Ib, IIb and IIIb) placed on leaves of *P.guajava* from non-crude oil polluted site. Very low-density lipoprotein (VLDL) is another blood fat that is as bad as LDL-C. It is one of the four major lipoprotein particles. It is considered a bad cholesterol which contains triglycerides [33-34]. The observed values of VLDL increased significantly in rats groups (Ia, IIa and IIIa) placed on leaves of *P. guajava* from crude oil polluted site against the respective rats groups (Ib, IIb and IIIb) placed on leaves of *P. guajava* from non-crude oil polluted site. High VLDL-C and triglyceride simply mean a very high risk of cardiovascular disease [33-34]. The high values of VLDL as observed in the present study could imply a very high risk of cardiovascular disease for rats placed on leaves of *P. guajava* from crude oil polluted site.

Table 3: Atherogenic indices of rats placed on *P. guajava* from polluted and non-polluted areas.

| | Group I | | Group II | | Group III | |
|---|---|---|---|---|---|---|
| Parameters | Ia | Ib | IIa | IIb | IIIa | IIIb |
| LDL-C/HDL-C | 0.90 | 0.49 | 0.79 | 0.24 | 0.58 | 0.13 |
| CRR | 2.49 | 1.95 | 2.29 | 1.62 | 1.89 | 1.51 |
| AC | 1.49 | 0.95 | 1.29 | 0.62 | 1.12 | 0.51 |
| AIP | 0.46 | 0.35 | 0.40 | 0.27 | 0.44 | 0.28 |

*LDL-C= Low density lipoprotein cholesterol; HDL-C=High density lipoprotein cholesterol;* CRR= Cardiac risk ratio; AC= Atherogenic coefficient; AIP= Atherogenic index of plasma.

LDL-C/HDL-C ratio is an important parameter of risk assessment for dyslipidaemia [35]. Christine et al. [35] noted that LDL-C/HDL-C ratio is a stronger predictor of coronary heart disease. Total cholesterol /HDL-C and LDL-C/HDL-C ratios have been used as an indices of ischemic heart disease in men [36]. Both AC and AIP have been found to indicate atherogenic risk and are better predictors of cardiovascular risk than lipids alone [37]. AIP which is a mathematical relationship of TG and HDL-C, has been used effectively as an index for assessment of cardiovascular risk [38]. Atherogenic indices presented in Table 3 had LDL-C/HDL-C ratio ranged from 0.13 to 0.90; CRR ranged from 1.51 to 2.29; AC ranged from 0.51 to 1.49; and AIP ranged from 0.27 to 0.46. All the indices were high in rats placed on leave of *P. guajava* from crude oil polluted site against those placed on leaves of *P. guajava* from non-crude oil polluted site. This could imply higher exposure to risk of cardiovascular and heart diseases.

Table 4: Level of reproductive hormones of rats placed on *P. guajava* from polluted and non-polluted areas.

| | Group I | | Group II | | Group III | |
|---|---|---|---|---|---|---|
| Parameters | Ia | Ib | IIa | IIb | IIIa | IIIb |

| | | | | | | |
|---|---|---|---|---|---|---|
| Luteinizing hormone (miu/ml) | 0.40±0.02 | 0.12±0.01* | 0.47±0.03 | 0.26±0.01* | 0.58±0.06 | 0.28±0.04* |
| Testosterone (ng/ml) | 1.56±0.13 | 0.29±0.14* | 1.78±0.17 | 0.54±0.18* | 1.89±0.12 | 0.98±0.10* |

Results are mean and standard deviation of triplicate determinations. Values of "b" subgroup asterisked against those of "a" subgroup under a main group on the Table are statistically significant at $p<0.05$.

Luteinizing hormone (LH) is one the gonadotropins that stimulate the gonads both in testes of male and ovaries of female [39-40]. Luteinizing hormone stimulates the synthesis as well as the secretion of testosterone with the help of its receptors that bind to leydig cells [40-41]. Both hormones are important tools of reproduction and have steroid nucleus [41]. Luteinizing hormone ranged from 0.12 to 0.58 miu/ml while testosterone ranged from 0.29 to 1.89 ng/ml (Table 4). Levels of luteinizing and testosterone hormones as observed in the present study, increased significantly ($p<0.05$) in rat groups Ia, IIa and IIIa when compared to those of rat groups Ib, IIb and IIIb. The observed increase in the hormones could be linked to increased cholesterol in rats placed on *P. guajava* leaf sample from crude oil polluted.

## CONCLUSION

Rats groups placed on *P. guajava* leaf sample from crude oil polluted area showed marked degeneration in lipid profile and atherogenic indices against rat groups placed on *P. guajava* leaf sample from non-crude oil polluted area. There is to sensitise those in the act of herbalism to be aware of where they harvest the plants they use as raw materials. This study has shown the variation in body weight, lipid profile and selected reproduction hormones in rats given *P. guajava* leaves from crude oil polluted and non-crude oil polluted areas.

**Ethical Approval:**

As per international standard or university standard ethical approval has been collected and preserved by the authors.

## REFERENCES


[1]. World Health Organization (WHO). Promotion and development of traditional medicine. Tech. Rep. Series. 1978; p.622.

[2]. Sofowora A. Medicinal plants and traditional medicine in African. Spectrum Books Ltd, Ibadan, Nigeria, 1993, pp. 191-289.

[3]. Duru M, Amadi B, Agomuo E, Eze A. Chemical profile of an anti-malarial concoction "Udu" used in Umunchi autonomous community in Isiala Mbano L.G.A of Imo State, Nigeria. Journal of Emerging Trends in Engineering and Applied Science**.** 2012; 3(3): 444-447.

[4]. Amadi BA, Duru MKC, Agomuo EN. The chemical profiles of leaf, stem, and flower of *Ageratum conyzoides*. Asian Journal of Plant Sciences and Research.2012; 2(4):428-43

[5]. Agomuo EN, Onyeike EN, Anosike EO, Duru MKC. Effects of *Tetrapleura tetraptera* on lipid profiles and mineral concentration of male, female, pregnant and lactating albino rats. Intraspecific Journal of Biochemistry and Biotechnology. 2014; 1(1):001-007.

[6]. Arukwe U, Amadi BA, Duru MKC, Agomuo E, Adindu EA, Odika PC, Lele KC, Egejuru L, Anudike, J. Chemical composition of *Persea americana* leaf, fruit and seed. International Journal of Research and Reviews in Applied Sciences. 2012; 11(2):355.
[7]. Duru MKC, Amadi BA, Agomuo EN, and Njoku VO. The effect of fresh, oven dried uncooked and cooked seeds of *Buchholza coriacea* on weight of visceral organs, tissue lipid and testosterone. Proceedings of the 37th Annual International Conference, Workshop & Exhibition of Chemical Society of Nigeria.2014; pp 127-132.
[8]. Duru M, Amadi B, Njoku V. Evaluation of some chemical constituents of "ji-otor" and "ntubiri ikpa" traditional foods of Ikwerre ethnic national in Nigeria. Proceedings of the 36th Annual International Conference, Workshop & Exhibition of Chemical Society of Nigeria.2013; Vol.1.pp 38-44.
[9]. Amadi BA, Arukwe U, Duru MKC, Amadi CT, Adindu EA, Egejuru L, Odika PC. Phytonutrients and antinutrients screening of *D.edulis* fruits at different maturation stages. Journal of Natural Product Plant Resourse. 2012; 2 (4):530-533.
[10]. Taylor JLS, Rabe T, McGaw LJ, Jäger AK, van Staden J. Towards the scientific validation of traditional medicinal plants. Plant Growth Regulators.2001; 34: 23-37.
[11]. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigeria medicinal plants. African Journal of Biotechnology. 2005; 4(7):685-688. `
[12]. Agomuo EN, Amadi BA, Duru MKC. Some biochemical studies on the leaves and fruits of *Persea americana.* International Journal of Research and Reviews in Applied Sciences. 2011;11(3):565-569.
[13]. Duru MKC, Arukwe U, Amadi, BA. Bioactive constituents and macronutrients composition of anti-malarial concoction used in Umunchi village in Isiala Mbano L.G.A of Imo State, Nigeria. International Science Research Journal. 2011;3: 61-64.
[14]. Duru M, Agomuo E, Amadi B, Iheanacho A, Nutritional evaluation and some biochemical studies *on Ageratum conyzoides* using its different par*ts.* Proceedings of the 35th Annual International Conference, Workshop & Exhibition of Chemical Society of Nige*ria.* 2012;1:372-379.
[15]. Agomuo E, Duru M, Amadi B, Amadi P, Ugwokaegbe P. Effect of caffeine on some selected biochemical parameters using rat model. Advances in Biology; 2017. [Article ID 9303276, 8 pages] Available:https:/ /doi.org/10.1155/2017/9303276
[16]. Duru M, Amadi C, Ugbogu A, Eze A, Amadi B. Phytochemical, vitamin and proximate composition *of Dacryodes edulis* fruit at different stages of maturation. Asian Journal of Plant Science and Research. 2012;2(4):437-441.
[17] Nwachukwu MI, Duru MKC, Amadi BA, Nwachukwu IO. Comparative evaluation of phytoconstituents, antibacterial activities and proximate contents of fresh, oven dried uncooked and cooked samples *of Buchholzia coriacea* s*eed* and their effects on hepatocellular integrity International Journal of Pharmaceutical Science Invention. 2014; 3(6):41-4.
[18]. Nwachukwu MI, Duru MKC, Nwachukwu IO. Antifungal properties and effect of fresh, oven dried uncooked and cooked seeds *of Buchholzia coriacea* on haematology and kidney. Elixir Food Science.2013;64:19350-19356.
[19]. Gutierrez RMP, Mitchell S, Solis RV. *Psidum guajava*. A review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol. 117:1-27.
[20]. Hawrelak J. Medicinal herb monograph: Guava (*Psidium guajava*). J. Aust. Traditional Med. Soc. 2003; 9: 25-29.
[21]. Adeyemi OS, Akanji MA, Ekanem JT. Ethanolic extract of *Psidium guajava* influences protein and bilirubin level Trypanosoma brucei brucei infected rats. Journal of Biological Sciences. 2012; 12 (2): 111-116.
[22]. Offor CE, Okoro JA, Ibiam UA, Nwangwu SCO. Effect of ethanol leaf-extract of

*Psidium guajava* on lipid profile. Global Journal of Pharmacology. 2015; 9(1):77-80.
[23]. Dasimeokuna P, Eze A, Anudike J. Comparative effect of two similar medicinal plants from polluted and non-polluted areas on body weight, lipid profile and some reproductive hormones. Intraspecific Journal of Biochemistry and Biotechnology. 2019; 6:013-020.
[24]. National Institute of Health. "Guide for the care and use of laboratory animals". U.S. Department of Health Education and Welfare. Washington D.C: NIH Publication, 1985; pp.85-123.
[25]. Duru M, Amadi B, Ugbogu A, Adindu E. Effect of "udu", an antimalarial herbal preparation on visceral organ weight and blood lipid profiles in Wistar rats. 2014; JPCS 8:1-7.
[26]. Burnstein MA, Sammille J. A rapid determination of cholesterol bound to A and B-lipoprotein. Clin. Chem. Acta. 1960; 5: 601-609.
[27]. Duru MK, Amadi BA, Amadi CT, Ugbogu AE, Onuoha NL. Assessment of "nduduagworagwo", a traditional recipe of akokwa people in Ideato North L.G.A of Imo State, Nigeria on body weight and some biochemical parameters. Continental J. Food Science and Technology, 2013; 7 (1): 15 – 21.
[28]. Martirosyan DM, Miroshnichenko LA, Kulokaw SN, Pogojeva AV, Zoloedov VI. Amaranth oil application for heart disease and hypertension lipid health. Dis. 2007;6:1.DOI: 10.1186/1476-511x-6-1.
[29]. Dobiasova M, Frohlich J. The plasma parameter log (TG/HDL-C) as atherogenic index; correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein depleted plasma (FERHDL)". Clin Biochem. 2001;34:583-588.
[30]. Raji Y, Salman TM, Akinsomisoye SO. Reproductive functions in male rats treated with methanolic extracts of *Aistonia boonei* stem bark. Afri.J. Biomed. Res.2005; 8(2):105-111.
[31]. Brehm A, Pfeiler G, Pacini G, Vierhapper H, Roden M. Relationship between serum lipoprotien ratios and insulin resistance in obesity. Clin. Chem. 2004;50:2316-2322.
[32]. Lipid Research Clinics Programme. The lipid research clinics coronary primary prevention trial results. 1: reduction in the incidence of heart disease. JAMA. 1984; 251:351-364.
[33]. Glew RH. Lipid metabolism II: Pathways of metabolism of special lipids. In: Devlin TM (ed) Textbook of biochemistry with clinical correlations, 6th edn. Wiley Liss, New Jersey. 2006; 695-741.
[34]. Ugbogu AE, Okezie E, Uche-Ikonne C, Duru M, Atasie OC. Toxicity evaluation of the aqueous stem extracts of *Senna alata* in wistar rats. American Journal of Biomedical Research, 2016; 4 (4): 80-86.
[35]. Christine MG, Tosca LZ, Richard JW, Sudeep S, Dimple A, Matthew JS, Jeff SV, Maria LF. Maintenance of the LDL cholesterol:HDL cholesterol ratio in an elderly population given a dietary cholesterol challenge.2005; Human Nutrition and Metabolism. 2793-2798.
[36]. Isabelle L, Benott L, Charles C, Agnes P, Bernard C, Jean B, Gilles RD, Jean-Pierre D. Total cholesterol/HDL cholesterol ratio ves LDL cholesterol/HDL cholesterol ratio as indices of ischemic heart disease risk in men. 2001; Arch Intern Med. 161: 2685-2692.
[37]. Nimmanapalli HA, Kasi AD, Davapatla PK, Nuttakki V. Lipid ratios, atherogenic coefficient and atherogenic index of plasma parameters in assessing cardiovascular risk in type 2 diabetes. 2016; 4(7):2863-28
[38]. Dobiasova, M. AIP-atherogenic index of plasma as a significant predictor of cardiovascular risk: from research to practice. Vnitr. Lek. 2006, 52: 64–71.
[39]. Duru M, Nwadike C, Ezekwe A, Nwaogwugwu C, Eboagwu I, Odika P, Njoku S,

Chukwudoruo C. Evaluation o.f nutritional, anti-nutritional and some biochemical studies on *Pleurotus squarrosulus* (Mont.) singer using rats. African Journal of Biochemistry Research. 2018;12 (2):7-27.
[40]. Walker S, McMahon D. Biochemistry demystified, a self- teaching guide. McGraw Hill USA.2008; pp.82-87.
[41]. Duru M, Eboagwu I, Kalu W, Odika P. Nutritional, anti-nutritional and biochemical studies on the oyster mushroom, *Pleurotus ostreatus*. EC Nutrition.2019; 14 (1): 36-59.