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Hypolipidemic properties of the methanol leafy extracts of *Pupalia lappacea and Morinda lucida* on diet-induced lipidemic albino wister rats: A comparative analysis

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Abstract
Introduction
Comparative hypolipidemic properties of methanol extracts of Pupalia lappacea and Morinda lucida *on diet-induced lipidemic rats were studied.*
Methodology: *Thirty (30) rats weighing between 180g-200g ± 20g were assigned into six (6) groups having five (5) animals per group according to their weight (X ±20 g). Group A was the normal control and received normal rat pellets only, without extract and cholesterol. Group B received 1000mg/kg of cholesterol and normal rat pellets without extract. While groups C, D, E received 10mg/kg, 100mg/kg, 1000mg/kg of the plant extract plus 1000mg/kg of cholesterol and normal rat pellets, respectively in P.* lappacea *model, they received 100mg/kg, 2000mg/kg and 5000mg/kg of the plant extract plus 1000mg/kg of cholesterol and normal rat pellets, respectively in M.lucida model. Group F received 5mg/kg of Fenofibrate plus 1000mg/kg of cholesterol and normal rat pellets in both cases. The experiment lasted for 28days. On the 29th day, the animals were bled through retro-orbital puncture under ether anaesthesia after an overnight fast and the sera separated by centrifugation. Lipid profile (cholesterol, high density lipoprotein-HDL, low density lipoprotein-LDL and triglyceride-TAG) was analyzed according to standard methods. SPSS analytical software windows version 15 was used for statistical analysis and one way analysis of variance (ANOVA) determine differences between means, followed by Tukey's post-hoc comparisons. $P < 0.05$ was considered significant. Comparison of the post-treatment and baseline values was used as an index of hypolipidemic activities.*
Result: *There were post induction increases and post-treatment decreases in all the lipid components. The baseline and post treatment values of cholesterol in groups D ($p = 0.639$), E ($p = 0.080$) and E ($p 0 = .058$) in* P. lappacea *and M.lucida, respectively and the LDL values across the groups ($p > 0.05$) in* P. lappacea and in *groups D ($p = 0.413$), E ($p=0.382$) and F ($p = 0.938$) in M.lucida showed no significant difference. The baseline and post treatment TAG values in groups D ($p = 0.170$), E ($p = 0.340$), F ($p = 0.077$) and D ($p = 0.848$), E ($p = 0.122$) and F ($p = 0.077$) in* P. lappacea *and M.lucida, respectively had no significant change after treatment than at baseline.* ***Conclusion***
Both plants extracts possess hypolipidemic activities in all the lipid components albeit at different degrees.

Key words: Hypolipidemia, High Density Lipoprotein, Low Density Lipoprotein, Cardiovascular Diseases, Phytotherapeutic Agents. ***2,*for correspondence***

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I. Introduction

Recently, the prevalence of non-communicable diseases (NCDs), such as cardiovascular diseases (CVDs) and chronic obstructive pulmonary diseases (COPDs) have become an emerging pandemic globally with developing countries recording higher rates (Terzic and Waldman 2011). The World Health Organization (WHO, 2013) estimates that by 2020, NCDs will account for 80 percent of the global burden of disease, causing seven out of every ten deaths in developing countries, about half of them premature deaths of children under the age of 7 (WHO, 2013).

NCDs deaths worldwide exceed all communicable diseases death and represent an emerging global health threat (Alwan *et al* 2001). Majority of the deaths occur in low and middle-income countries where the numbers of people affected by NCDs are growing. The health systems are often not equipped to respond effectively. In developing countries where intercultural pollution has affected nutritional behaviours adversely, cardio-vascular diseases and other nutrition- related diseases are on the increase. The resultant effect is diseases that were aliens to Nigeria culture are now very common among all ages and sexes. In a culture characterized with illiteracy, fragile economic base and poor public health policies, the impact of NCDs has recently been forced into mass consciousness as the death tolls associated with these diseases affect virtually every family. There is an urgent need to look inwards and begin to manage the diseases with locally available resources. The plant kingdom holds a strong promise to this quest as many tropical plants have been reported to be efficacious in the management of tropical disease(Inya-Agha et al 2006, Igoli *et al*., 2006) , if scientists can direct research beam to herbal plants, with the aim to establishing their phytochemical and nutritional compositions that may be harnessed in ethno medicine.

In Nigeria as with other developing countries of the world, the morbidity and mortality of NCDS have become more glaring(Opadijo 2004) owing to a lot of factors, including socio-economy, inadequate primary healthcare delivery and illiteracy, to mention but a few. With the prevailing economic recession in Nigeria, access to conventional synthetic drugs will continue to be classic, exclusively within the reach of the privileged of the society. This will shift the disease burden to the poor and less privileged unless alternative disease management through non-synthetic means is explored. There is a strong need to explore alternative means of cardio-vascular and related diseases management using natural, easily available and cost effective, safe resources. *P.lapecia* and *M.lucida* which have been used in folk remedies and are reported to have a broad range of therapeutic and prophylactic effects (Asuzu *et al.,* 1990, Olajide *et al*., 1999 Joppa *et al.,* 2008, Oduola *et al.,* 2010, Domekouo *et al.,* 2016).are good candidates for further investigations into their effects on cardio-vascular diseases risk factors such as hyperlipidemia. This study investigated the lipid lowering potentials of the methanol extracts of *P. lappacea* and *M.lucida* in albino Wister rats on cholesterol- rich diet.

II. Materials and Methods

2.1 Collection, identification and treatment of plant samples

Fresh leaves of *P. lappacea,* and *M.lucida* plants were randomly collected from the bush and fallow lands in the towns of Egede and Enugu Ngwo, both in Udi L.G.A of Enugu State. They were identified by a plant taxonomist in the Department of Plant science and Biotechnology, University of Nigeria Nsukka. They were washed with clean tap water, allowed to drain for 15mins in a plastic sieve and dried at room temperature for seven (7) days. The dried samples were pulverized into a homogeneous texture of 60µ using a laboratory hammer mill.

Preparation of methanol extracts of the four plant samples

Methanol extract of each plant sample (*P. lappacea*, *M. lucida*) were prepared by cold maceration as described by Ibeziem *et al* (2012) with slight modifications. The extracts were screened for phytoconstituents following standard procedures (Harborne 1984, Trease and Evans 2002).

Experimental Design and Conduct

Thirty (30) rats weighing between 180g-200g ± 20g obtained from the animal house unit of the College of Medicine, University of Nigeria Enugu Campus, were assigned into six (6) groups having five (5) animals per group according to their weight (X ±20 g), after oral acute toxicity was determined in rat as described by Lorke (1983). Group A was the normal control and received normal rat pellets only, without extract and cholesterol. Group B received normal rat pellets and 1000mg/kg of cholesterol but without extract. While groups C, D, E received 10mg/kg, 100mg/kg, 1000mg/kg of the plant extract plus 1000mg/kg of cholesterol, respectively in *P.*

lappacea model, they received 100mg/kg, 2000mg/kg and 5000mg/kg of the plant extract plus 1000mg/kg of cholesterol, respectively in *M.lucida* model. Group F received 5mg/kg of Fenofibrate plus 1000mg/kg of cholesterol in both cases. The experiment lasted for 28days. On the 29th day, the animals were bled through retro-orbital puncture under ether anaesthesia after an overnight fast. The blood samples were allowed to stand undisturbed to clot and retract; the sera were separated by centrifugation and stored frozen for lipid profile. All experimental protocols and animal handling were in compliance with the international guidelines for experiments involving the use of animals as reported in McGrath *et al* (2010).

Biochemical analysis

The parameters tested for were; Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Triglyceride (TG), Low Density Lipoprotein Cholesterol (LDL-C) – calculated. Total cholesterol was analysed using the enzymatic method of Fredrickson (Fredrickson, 1967), HDL cholesterol by phophotungstate method of Richmond (Richmond, 1973) and triglyceride by GPO-PAP method of Trinder (Trinder, 1969) and LDL cholesterol values were calculated using the empirical Friedewald equation (Friedwald, 1972). Reagent kits for the analyses were the products of Randox laboratories Limited, United Kingdom. Tests were carried out following manufacturer's manual

Data analysis

Results were expressed as mean ± standard error of mean. The analysis was carried out on SPSS analytical software windows version 15. Differences between means were determined by the one way analysis of variance (ANOVA) followed by Tukey's *post-hoc* comparisons. $P < 0.05$ was considered significant. Comparison of the post-treatment and baseline values was used as an index of hypolipidemic activities

III. Results And Discussion

The pytoconstituents identified were flavonoids (++, +++), tannins (++, ++), alkaloid (++, +++), saponnins(+,+), phenols (++, ++++), glycosides (++,++),terpenoids (++,++++) in *P. lappacea* and *M.lucida*, respectively. *M.lucida* extract contained alkaloid, phenols and terpenoids more abundantly than *P. lappacea.* The extracts had phytoconstituents of medicinal importance and the findings were in line with those of Adesogan *et al* (1984), Rath (1995), Trease and Evans (2002), Olajide *et al* (1999) which showed that alkaloids, glycosides, saponins, flavonoids, tannins, terpenoids, steroids were present in the leaf, stem, bark and root of the plants under study.

The extracts had a wide therapeutic window as no death was recorded in the dose- ranges used in the acute toxicity test but the *M.lucida* extract had a wider margin of safety(5000mg/kgbwt*)* than *P. lappacea* (1000mg/kgbwt)

The rise in all components of lipid profile above the baseline values after induction indicated the success of the diet-induced lipidemia. This is in agreement with other studies elsewhere during which cholesterol–enriched diets were used to induce hyperlidemia in rats (Annamária *et al* 2003, Giricz *et al* 2009, Fatma *et al*., 2019).

Though the extracts caused a significant decrease in cholesterol and low-density lipoprotein (LDL) values of the diet-induced lipidemia after treatment when compared with the control group (A), non-treated (B) and the baseline values, the effects vary between the two plants. The baseline and post treatment cholesterol values in *P. lappacea* (table1) showed significant differences only in groups C ($p = 0.006$) and F ($p < 0.001$) but the baseline and post treatment cholesterol values in *M.lucida* (table 2) showed significant difference in groups C ($p < 0.001$), D ($p = 0.005$) and F ($p = 0.001$). At the maximum concentrations of the extracts (1000mg/kgbwt and 5000mg/kgbwt) for *P. lappacea* and *M.lucida* respectively, the post treatment effects on cholesterol were more pronounced in *M.lucida* extract when compared to control A (79.40±5.22mg/dl against 80.00±6.44mg/dl) than in *P. lappacea* (82.00±6.12mg/dl against 80.00±6.44mg/dl).

Table1: **Effects of *P. lappacea* Methanol Leaf Extracts on Cholesterol (mg/dl) of Diet-induced Hyperlipidemic Rats**

Group	Baseline M±SD	After Treatment M±SD	T	p-value
- A (not induced)	63.20±2.39	80.00±6.44	-	-
- B (not treated)	68.80±4.66	126.40±3.91	-	-
- C (10mg/kg^3)	70.60±10.09	100.80±5.07	5.327	.006^c
- D (100mg/kg^3)	81.40±9.94	83.60±6.23	.507	.639^b
- E (1000mg/kg^3)	70.60±6.99	82.00±6.12	2.334	.080^b
- F (Std. drugs)	66.80±5.36	85.80±5.50	8.253	.001^c

Superscripts: a = groups with significantly lesser cholesterol level after treatment than at baseline; b = groups with no significant change after treatment than at baseline; c = groups with significantly higher cholesterol level after treatment than at baseline; n = 5 per group

Table 2: **Effects of *M.lucida* Methanol Leaf Extracts on Cholesterol (mg/dl) of Diet-induced hyperlipidemic Rats**

Group	Baseline M±SD	After Treatment M±SD	T	p-value
- A (not induced)	63.20±2.39	80.00±6.44	-	-
- B (not treated)	68.80±4.66	124.40±3.51	-	-
- C (100mg/kg^3)	64.60±5.18	101.00±8.66	11.962	< .001^c
- D (2000mg/kg^3)	65.00±4.47	86.80±4.60	5.548	.005^c
- E (5000mg/kg^3)	65.00±7.97	79.40±5.22	2.635	.058^b
- F (Std. drugs)	66.80±5.36	85.80±5.50	8.253	.001^c

Superscripts: a = groups with significantly lesser cholesterol level after treatment than at baseline; b = groups with no significant change after treatment than at baseline; c = groups with significantly higher cholesterol level after treatment than at baseline; n = 5 per group

While the baseline and post treatment LDL values (tables 3 and 4) showed no significant difference across the groups except for group C (p = 0.026, p = 0.019) in *lappacea* and *M.lucida* respectively, C (p = 0.019) in *M.lucida* had LDL level after treatment significantly higher than the baseline, but at the maximum concentrations of the extracts (1000mg/kgbwt and 5000mg/kgbwt) for *P. lappacea* and *M.lucida*, respectively, the post treatment effects on LDL were more pronounced in *M.lucida* extract when compared to group B (15.00 ± 1.00mg/dl against 71.00±6.56 mg/dl) than in *P. lappacea* (16.80 ± 2.59mg/dl against 71.00±6.56 mg/dl). When the post-treatment values at maximum concentrations of the extracts were compared with the group A baseline value, *M.lucida* extract also showed more activity (15.00 ± 1.00 mg/dl against 15.80±3.11mg/dl) than *P. lappacea* (16.80±2.59 mg/dl against 15.80±3.11 mg/dl)

Table 3: **Effects of *P. lappacea* Methanol Leaf Extracts on LDL (mg/dl) of Diet-induced hyperlipidemic Rats**

Group	Baseline M±SD	After Treatment M±SD	T	p-value
- A (not induced)	15.80±3.11	17.20±2.28	-	-
- B (not treated)	17.40±2.41	71.00±6.56	-	-
- C (10mg/kg^3)	17.60±2.70	18.00±1.58	.250	.815^b
- D (100mg/kg^3)	21.00±1.87	17.20±2.77	-2.598	.060^b
- E (1000mg/kg^3)	23.20±2.28	16.80±2.59	-3.441	.026^a
- F (Std. drugs)	18.80±1.92	19.00±3.87	.083	.938^b

Superscripts: a = groups with significantly lesser LDL level after treatment than at baseline; b = groups with no significant change after treatment than at baseline; c = groups with significantly higher LDL level after treatment than at baseline; n = 5 per group

Table4: **Effects of *M.lucida* Methanol Leaf Extracts on LDL (mg/dl) of Diet-induced hyperlipidemic Rats**

Group	Baseline M±SD	After Treatment M±SD	T	p-value
- A (not induced)	15.80±3.11	17.20±2.28	-	-
- B (not treated)	17.40±2.41	71.00±6.56	-	-
- C (100mg/kg^3)	16.60±2.41	21.60±1.52	3.835	.019^c
- D (2000mg/kg^3)	15.80±3.11	17.80±2.39	.913	.413^b
- E (5000mg/kg^3)	16.60±2.79	15.00±1.00	-.981	.382^b
- F (Std. drugs)	18.80±1.92	19.00±3.87	.083	.938^b

Superscripts: a = groups with significantly lesser LDL level after treatment than at baseline; b = groups with no significant change after treatment than at baseline; c = groups with significantly higher LDL level after treatment than at baseline; n = 5 per group

At the maximum concentrations of the extracts (1000mg/kgbwt and 5000mg/kgbwt) for *P. lappacea* and *M.lucida*, respectively the post treatment effects on HDL were more pronounced in *P. lappacea* extract when compared to group B (68.20±5.93 mg/dlagainst 14.20±2.28 mg/dl) than in *M.lucida* (62.60±2.97 mg/dl against 14.20±2.28 mg/dl). When the post-treatment values at maximum concentrations of the extracts were compared with the group A baseline value, *P. lappacea* extract also showed more activity (68.20±5.93 mg/dlagainst 86.20±4.92 mg/dl) than *M.lucida* (62.60±2.97 mg/dl against 86.20±4.92 mg/dl)

Table 5: Effects of *P. lappacea* Methanol Leaf Extracts on HDL (mg/dl) of Diet-induced hyperlipidemic Rats

Group	Baseline M±SD	After Treatment M±SD	T	p-value
- A (not induced)	86.20±4.92	75.40±6.54	-	-
- B (not treated)	79.60±3.29	14.20±2.28	-	-

- C (10mg/kg^3)	78.40±4.83	62.20±2.86	-11.631	< .001[a]
- D (100mg/kg^3)	81.40±5.13	63.00±1.41	-7.025	.002[a]
- E (1000mg/kg^3)	71.00±2.24	68.20±5.93	-1.095	.335[b]
- F (Std. drugs)	82.40±2.30	53.20±5.45	-12.519	< .001[a]

Superscripts: a = groups with significantly lesser HDL level after treatment than at baseline; b = groups with no significant change after treatment than at baseline; c = groups with significantly higher HDL level after treatment than at baseline; n = 5 per group

Table 6: **Effects of *M.lucida* Methanol Leaf Extracts on HDL (mg/dl) of Diet-induced hyperlipidemic Rats**

Group	Baseline M±SD	After Treatment M±SD	T	p-value
- A (not induced)	86.20±4.92	75.40±6.54	-	-
- B (not treated)	79.60±3.29	14.20±2.28	-	-
- C (100mg/kg^3)	90.40±3.85	43.20±4.44	-12.923	< .001[a]
- D (2000mg/kg^3)	88.20±2.68	55.20±3.70	-11.892	< .001[a]
- E (5000mg/kg^3)	85.40±7.99	62.60±2.97	-6.290	.003[a]
- F (Std. drugs)	82.40±2.30	53.20±5.45	-12.519	< .001[a]

Superscripts: a = groups with significantly lesser HDL level after treatment than at baseline; b = groups with no significant change after treatment than at baseline; c = groups with significantly higher HDL level after treatment than at baseline; n = 5 per group

At the maximum concentrations of the extracts (1000mg/kgbwt and 5000mg/kgbwt) for *P. lappacea* and *M.lucida*, the post treatment effects on TAG were more pronounced in *P. lappacea* extract when compared to group B (95.80±12.01mg/dl against 133.40±3.85 mg/dl) than in *M.lucida* (96.40±5.18 mg/dl against 133.40±3.85 mg/dl). When the post-treatment values at maximum concentrations of the extracts were compared with the group A baseline value, *P. lappacea* extract also showed more activity (95.80±12.01 mg/dl against 94.80±4.15 mg/dl) than *M.lucida* (96.40±5.18 mg/dl against 94.80±4.15 mg/dl)

Table7: **Effects of *P. lappacea* Methanol Leaf Extracts on TAG (mg/dl) of Diet-induced hyperlipidemic Rats**

Group	Baseline M±SD	After Treatment M±SD	T	p-value
- A (not induced)	94.80±4.15	94.20±5.67	-	-
- B (not treated)	95.60±4.16	133.40±3.85	-	-
- C (10mg/kg^3)	86.00±5.70	95.00±3.61	3.087	.037[c]
- D (100mg/kg^3)	87.80±2.86	92.60±6.62	1.672	.170[b]
- E (1000mg/kg^3)	90.80±3.11	95.80±12.01	1.083	.340[b]
- F (Std. drugs)	94.00±3.39	101.40±6.99	2.369	.077[b]

Superscripts: a = groups with significantly lesser TAG level after treatment than at baseline; b = groups with no significant change after treatment than at baseline; c = groups with significantly higher TAG level after treatment than at baseline; n = 5 per group

Table 8: **Effects of *M.lucida* Methanol Leaf Extracts on TAG (mg/dl) of Diet-induced hyperlipidemic Rats**

Group	Baseline M±SD	After Treatment M±SD	T	p-value
- A (not induced)	94.80±4.15	94.20±5.67	-	-
- B (not treated)	95.60±4.16	133.40±3.85	-	-
- C (100mg/kg^3)	92.60±5.98	104.60±6.77	3.029	.039[c]
- D (2000mg/kg^3)	91.60±3.65	92.40±6.19	.204	.848[b]
- E (5000mg/kg^3)	92.00±2.74	96.40±5.18	1.956	.122[b]
- F (Std. drugs)	94.00±3.39	101.40±6.99	2.369	.077[b]

Superscripts: a = groups with significantly lesser TAG level after treatment than at baseline; b = groups with no significant change after treatment than at baseline; c = groups with significantly higher TAG level after treatment than at baseline; n = 5 per group

These observed effects probably could be due to the presence of the identified phytoconstituents. Alkaloids, glycosides, saponins and flavonoids are known to reduce serum lipid level in animals (Asghar *et al*., 2018, Semerdjieva and Zhaljazkov 2019). Literature showed that saponins may lower cholesterol by preventing its absorption after it has been excreted in the bile. saponins could do this by binding to bile salts or promoting the binding of bile salt to polysaccharides in dietary fibre, causing a reduction in enterohepatic circulation of bile acids and increase the faecal excretion (Rotimi *et al*., 2011). The usage of diet with high saponin content is also suggested to reduce heart diseases (Oakenfull, 1981, Hostettman and Marston, 1995). Flavonoids are water soluble polyphenolic molecules with antioxidants activity which has many beneficial effects on cardiovascular system (Evans, 1989). Epidemiological studies have illustrated that heart diseases could be managed with flavonoid intake (Peterson *et al*., 2012). Flavonoids prevent the oxidation of low density lipoprotein, lowers the blood level of cholesterol and triglycerides thereby reducing the risk for development of atherosclerosis

(Subramani and Casimir, 2002). Phytosterols are reported to displace intestinal cholesterol absorption from the intestine (Ikeda and Sugano, 1998; Demonty *et al*., 2009).

IV. Conclusion

This study has proved that the extracts of the two plants are good phytotherapeutic agents in lowering blood lipid and therefore should be recommended as a medicament in the management of cardiovascular diseases associated with hyperlipidemia. Given the high cost of using synthetic drugs in cardiovascular disease management, and the undesirable side effects, the use of the plants extracts is a welcome development. However, because of different degrees of effects of the two plants extracts on some lipid components there is a need for further investigations on the combined effects-synergistic or antagonistic, so that the hypolipidemic properties of the plants will be maximally utilized in CVDs management.

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