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THE SYNERGISTIC EFFECT OF ETHANOL LEAF EXTRACT OF *ANNONA MURICATA* AND *ARTOCARPUS HETEROPHYLLUS* ON HIGH FAT DIET INDUCED HYPERLIPIDAEMIA IN WISTAR ALBINO RATS

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ABSTRACT

The antihyperlipidaemic and antihypertensive effects of ethanol leaf extract of *Annona muricata*, *Artocarpus heterophyllus* and combined ethanol leaf extract of *Annona muricata* and *Artocarpus heterophyllus* on male wistar albino rats were studied. Forty eight (48) rats were given high fat diet (HFD) to induce hyperlipidaemia. The rats were treated with 200mg/kg body weight of *A. muricata* and *A. heterophyllus*, 100 and 200mg/kg body weight of combined ethanol leaf extract of *A. muricata* and *A. heterophyllus*. Ethanol leaf extract of *A. muricat*a and *A. heterophyllus* had a positive effect on the total cholesterol, triglyceride, and high density lipoprotein level of the diabetic rats. The effects of the combined ethanol leaf extract on the heart tissue oxidative stress markers of the rats were also studied. A decrease which is statistically significant at $p<0.05$ was observed in the malondialdehyde level (0.50±0.07 µmol/ml) of the research animals. The result showed that ethanol leaf extract of *A. muricata* and *A. heterophyllus* with their combined extract had antihyperlipidaemic effect on high fat diet-induced hyperlipidaemia in wistar albino rats.

KEYWORDS: Hyperlipedeamia, oxidative stress, antihyperlipidaemia, blood pressure, High fat diet, wistar albino rats.

INTRODUCTION

There has been a shift recently from orthodox medicine to natural medicine; this is because natural products have limited side effects. Though orthodox medicine has been beneficial to man but it is usually accompanied by different side effects ranging from liver toxicity, kidney problems and cancer. This has led to the huge attention given to natural products recently. It is known in African traditional medicine that herbs and roots are used in the treatment of various diseases and infections. Though this is practiced in African traditional medicine without the knowledge of the actual content of these plants and most times dosages given to individuals are usually through guess. Recent researches conducted on these plants have led to the discovery of their actual phytochemical content, bioactivity and toxicity. *A. muricata* and *A. heterophyllus* are one of such plants used traditionally in the treatment of diseases.

A.muricata is a green leafy plant commonly found in the tropics, it belongs to the annonaceae family.[1] It is a tree plant which grows up to 4 to 6 meters and bears a dark greenish fruit.[2] It produces fruits which are green in colour with the bark covered with spikes. The fruits are edible white pulp covered with black seeds which are not edible.[3] It has rich deposit of phytochemicals hence effective in the treatment of different diseases folklarly. Recently annopentocin A, annopentocin B, annopentocin C, cis-Annomuricin-D-one, trans-Annomuricin-D-one, Murihexocin A, Murihexocin A, Murihexocin A, Annocatalin which have anticancer activities were isolated from the leaves, fruits, seeds, pericarp, roots and stem of *A. muricata*. Longifolicin isolated from the seed of *A. muricata* has toxic effect on hepatoma cells.[4] The leaves are also rich in phytol a diterpene which is a potent anticancer compound.[5]

Jack fruit (*Artocarpus heterophyllus Lam*) is an edible plant from the family of Moraceae.[6] It is a plant commonly seen in southern Asia. It is commonly eaten in India and Bangladish. Its fruits are one of the biggest fruits ever existed. It produces a greenish fruit which has a rough skin with the inside whitish and succulent. The seeds are big and bean sharped; they are edible usually eaten as snacks in some Asian countries.[7]

The phytochemical contents of various parts of *A. heterophyllus* have been studied; study revealed that the seeds are rich in tannin and phenolic compounds.[8] The leaves are rich in phytochemicals and have various biological activities. Methanol extract of the leaves of *A. heterophyllus* possess anti-ulcer activity against indomethacin induced ulcers in rats.[9] Methanol and aqueous extract of the leaf of *A. heterophyllus* has also exhibited

anticonvulsant activity in wistar rats.[10] The leaves have shown antioxidant activity, it has the potential to reduce lipid peroxidation; this has been attributed to its high phenolic compound content.[11]

Hyperlipidaemia also known as dyslipidemia results from abnormal increase of blood lipids.[12] People who consume foods that contain too much fat stand a greater chance of developing hyperlipidaemia. Research has shown that high fat diet could lead to obesity (hyperlipidemia) and hence a risk factor to cardiovascular diseases.[13]

Increased blood pressure is commonly referred to as hypertension, it usually affects a person when the pressure of the blood flowing through the arteries is very high due to tiny arteries.[14] Cardiac arrest, coronary heart disease and stroke are linked to high blood pressure.[15] Risk factors to high blood pressure include life style (feeding, smoking), age (more in people aged 65 and above), pregnancy (preeclampsia), obesity, alcohol and excessive intake of sodium (table salt).[16] A systolic blood pressure greater than 140mm/Hg and a diastolic blood pressure greater than 90mm/Hg in humans is considered high blood pressure or hypertension.[14] [15]

MATERIALS AND METHODS

Chemicals

Collection and identification of plant material:

The leaves of *A. muricata* were collected at the Abuja park of University of Port Harcourt Choba, Rivers state Nigeria while the leaves of *A. heterophyllus* were collected from Ozuoba Obio/Akpo Local Government Area of Rivers State Nigeria. The plants were identified in the Herbarium of Department of Plant Science and Technology, University of Port Harcourt. The Plant samples were washed and air dried under shade. The dried samples were homogenized to fine powder and stored in sterile air tight bottles for the experimental work.

Experimental Animals

The total number of animals used for this experiment was forty eight (48). They were purchased and housed in the Pharmacology Department Animal house at Ofrima, Abuja Park of the University of Port Harcourt, Choba, Rivers state. The animals were acclimatized for seven days (7), after which they were fed with high fat diet and clean water. The Wistar albino rats weighed 150 to 200 and they were marked for easy identification. Forty eight rats

were grouped in to six different groups. Mortality rate was observed and the animals were grouped as follows:

Group 1: Normal control.

Groups 2: Hyperlipidemia control: High fat diet

Group 3: High fat diet + 100mg/kg body weight of the combined ethanol leaf extract

Group 4: High fat diet + 200mg/kg body weight of the combined ethanol leaf extract

Group 5: High fat diet + 100mg/kg body weight of ethanol leaf extract of *A. muricata*

Group 6: High fat diet + 100mg/kg body weight of ethanol leaf extract of *A. heterophyllus*

Preparation of Extract

A.Muricata and *A. heterophyllus* leaf extracts: The Leaves of *A .muricata* and *A. heterophyllus* were washed and shade dried, after which the leaf powder was prepared using home grinder/blender. Two hundred (200g) of the powdered *A.muricata* and *A. heterophyllus* leaves were weighed and soaked in 1000ml of 95% Ethanol for 48 hours after which they were sieved using a muslin cloth and afterwards filtered with Whatmann paper size 1. The filtrates were concentrated using Rotary Evaporator at 45^{o}C, the weights of the concentrates were taken and the percentage yield calculated and kept at 4^{o}C until usage.

Preparation of High fat diet

A high fat diet was prepared with 20% SUCROSE, 10% margarine (baking fat), 2.5% egg yolk and 67.5% finisher (animal feed).

Statistical analysis

All data were subjected to statistical analyses. Values are reported as Mean ± standard error of mean (SEM) while one way ANOVA was used to test for differences between treatment groups using Statistical Package for Social Sciences (SPSS) version 16. The results were considered significant at *p*-values of less than 0.05, that is, at 95% confidence level ($P<0.05$).

RESULTS

Table 1: The effect of ethanol leaf extract of *A. muricata*, *A. heterophyllus* and combined ethanol leaf extract of *A. muricata* and *A. heterophyllus* on the total cholesterol levels of male wistar albino rats.

Groups	Total cholesterol(Mmol/l)		Triglyceride (Mmol/l)		HDL (Mmol/l)	
	After 15 days	After 30 days	After 15 days	After 30 days	After 15 days	After 30 days
Normal control	1.70±.46^{b}	1.63±.38^{b}	0.38±.13^{b}	0.38±.06	0.55±0.48^{bc}	0.46±0.05^{bc}
Disease control	4.07±.72^{ac}	4.13±.75^{ac}	0.70±.05^{ac}	0.58±.16	0.41±0.12^{c}	51.02±0.07^{c}
HFD + 100mg combined Extract	1.90±.56^{b}	1.83±.15^{b}	0.53±.05	0.43±.11	0.32±0.13^{c}	0.33±0.05^{c}
HFD + 200mg combined Extract	1.70±.40^{b}	1.63±.21^{b}	0.56±.12	0.52±.03	0.58±0.09	0.50±0.07^{c}
HFD + 200mg *A. muricata*	1.23±.25^{b}	1.90±.10^{b}	0.51±.04	0.50±.02	0.70±0.37^{b}	0.58±0.17^{b}
HFD + 200mg *A. Heterophyllus*	1.93±.29^{b}	1.87±.42^{b}	0.51±.20	0.46±.07	0.71±0.37^{b}	0.69±0.16^{b}

Superscript “a” shows significant difference, (p<0.05) when Normal control (NC) is compared with other groups.

Superscript “b” shows significant difference, (p<0.05) when Disease control (DC) is compared with other groups.

Table 2: The effect of ethanol leaf extract of *A. muricata*, *A. heterophyllus* and combined ethanol leaf extract of *A. muricata* and *A. heterophyllus* on the blood pressure of male wistar albino rats.

Groups	Basal Systolic (mm/Hg)	Basal Diastolic (mm/Hg)	21 days after HFD Systolic (mm/Hg)	21 days after HFD Diastolic (mm/Hg)	15 days after treatment with PE Systolic (mm/Hg)	15 days after treating with PE Diastolic (mm/Hg)	30 days after treating with PE systolic (mm/Hg)	30 days after treating with PE diastolic (mm/Hg)
Normal control	151.0±10.00	76.00±5	154.00±5.29^{bc}	77.67±3.21	153.67±3.51^{bc}	80.33±1.527	152.00±1.00^{bc}	193.45±18.38
Disease control	155.00±4.35	76.67±4.93	208.33±3.78^{a}	79.33±0.577	213.00±2.00^{ac}	79.00±1.00	205.33±4.93^{a}	79.33±0.57
HFD + 100mg combined Extract	157.00±4.24	80.50±2.12	211.00±1.414^{a}	77.00±2.828	199.00±1.41^{ab}	86.00±7.07	207.00±9.89^{a}	78.00±2.64
HFD + 200mg combined Extract	147.67±5.85	77.67±2.309	208.00±4.358^{a}	79.00±1.00	196.33±3.79^{ab}	80.33±1.15	198.67±1.15^{a}	79.50±0.70
HFD+ 200mg *A. muricata*	151.33±7.23	79.00±1.00	212.67±2.51^{a}	80.33±1.52	197.33±4.62^{ab}	80.67±1.52	198.33±1.53^{a}	79.00±1.732
HFD + 200mg *A. Heterophyllus*	156.33±6.11	80.00±2.00	213.33±2.081^{a}	80.67±0.57	189.67±8.08^{ab}	76.00±4.582	199.00±1.00^{a}	80.00±1.00

Superscript “a” shows significant difference, (p<0.05) when Normal control (NC) is compared with other groups.

Superscript “b” shows significant difference, (p<0.05) when Disease control (DC) is compared with other groups.

Table 3: The effect of ethanol leaf extract of *A. muricata*, *A. heterophyllus* and combined ethanol leaf extract of *A. muricata* and *A. heterophyllus* on the oxidative stress parameters of male wistar albino rats.

Groups	MDA(µmol/ml)		SOD (u/ml)		GSH (µg/ml)		CAT (u/ml)	
	After 15 days	After 30 days	After 15 days	After 30 days	After 15 days	After 30 days	After 15 days	After 30 days
Normal control	1.27±o.06^{b}	1.25±0.06^{b}	0.36±0.03	0.32±0.01	0.13±.01	0.15±.03^{b}	10.73±1.58^{b}	12.20±.89^{b}
Disease control	3.55±0.42^{ac}	4.07±0.25^{ac}	0.50±0.06^{c}	0.49±0.13^{c}	0.06±.02^{c}	0.05±.03^{ac}	3.47±.76^{ac}	3.07±.96^{ac}
HFD + 100mg combined Extract	1.25±0.07^{b}	1.16±0.08^{b}	0.33±0.02^{b}	0.36±0.12	0.19±.07^{b}	0.20±.03^{b}	10.42±1.55^{b}	10.71±1.12^{b}
HFD + 200mg combined Extract	2.30±0.61^{b}	1.67±0.14^{b}	0.26±0.07^{b}	0.35±0.06	0.18±.04^{b}	0.17±.04^{b}	12.98±2.36^{b}	11.04±1.10^{b}
HFD + 200mg *A. muricata*	1.89±0.82^{b}	1.17±1.04^{b}	0.29±0.07^{b}	0.23±0.05^{b}	0.17±.05^{b}	0.19±.01^{b}	13.46±2.30^{b}	14.80±.71^{b}
HFD + 200mg *A. Heterophyllus*	1.54±0.28^{b}	1.62±0.21^{b}	0.23±0.02^{b}	0.18±0.06^{b}	0.16±.03	0.18±.03^{b}	11.24±.46^{b}	12.38±.45^{b}

Superscript “a” shows significant difference, (p<0.05) when Normal control (NC) is compared with other groups.

Superscript “b” shows significant difference, (p<0.05) when Disease control (DC) is compared with other groups.

DISCUSSIONS

Table 1 shows the effect of ethanol leaf extract of *A. muricata*, *A. heterophyllus* and combined ethanol leaf extract of *A. muricata* and *A. heterophyllus* on the lipid profile of the hyperlipideamic rats. Treatment with high fat diet led to an increase in the total cholesterol and triglyceride level and a reduction on the HDL level of the research animals. Treatment with 100mg/kg body weight of ethanol leaf extract of *A. muricata* and *A. heterophyllus* and different concentration (100 and 200 mg/kg) of the combined extract of *A. muricata* and *A. heterophyllus* led to a significant decrease (p<0.005) on the total cholesterol and triglyceride level of the animals while the HDL levels was increased as shown in table 1 above.

The effect of ethanol leaf extract of *A. muricata*, *A. heterophyllus* and combined ethanol leaf extract of *A. muricata* and *A. heterophyllus* on the blood pressure of the hyperlipideamic rats was studied. The result of this research shows no statistical significant effect (p<0.05) on the basal blood pressure of the rats. There was no effect on the diastolic blood pressure of all the animals after treatment for 30 days; however an increase was observed in the systolic blood pressure of the hyperlipideamic control rats on day 15 and day 30 respectively (213.00±2.00^{a} and 205.33±4.93). Treatment with the combined extract led to a slight reduction in the systolic blood pressure of the rats as shown in table 2 above.

Table 4 shows the effect of *A. muricata*, *A. heterophyllus* and combined ethanol leaf extract of *A. muricata* and *A. heterophyllus* on oxidative stress biomarkers of the heart tissue of the hyperlipideamic rats. Treatment with high fat diet led to an increase in the MDA level of the animals while there was a decrease in the GSH levels, SOD activities and catalase activities of the group 2 animals (hyperlipideamic groups) after 30 days. Treatment with 100mg/kg body weight of *A. muricata* and *A. heterophyllus* and different doses of combined ethanol leaf extract of *A. muricata* and *A. heterophyllus* led to a decrease in MDA level of the treated groups and an increase in the GSH levels, SOD and catalase activities of the treated groups.

DISCUSSION

The results of the present study indicate that oral administration of 100 mg/kg body weight of *A. muricata*, *A. heterophyllus*, 100 and 200 mg/kg body weight of combined ethanol leaf extract of *A. muricata* and *A. heterophyllus* led to a decrease in the total cholesterol and triglyceride levels of the treated animals while an increase was observed in the HDL levels of the treated animals. Hyperlipidaemia is characterised with increase in triglyceride and total cholesterol levels with a reduction on the high-density lipoprotein cholesterol (HDL-C) levels which could eventually lead to atherosclerosis and subsequently cardiovascular diseases.[17]

Studies have shown that excessive intake of high fat diet can lead to obesity and excessive fat deposits leading to atherosclerosis (narrowing of the arteries) which is a predisposing factor to high blood pressure. In the present study, increased systolic blood pressure observed before administration of the extracts can be attributed to the high fat diet given to the animals. This effect was reversed after oral administration of the plant extracts.

The effect of 100mg/kg body weight of *A. muricata*, *A. heterophyllus* and combined ethanol leaf extract of *A. muricata* and *A. heterophyllus* on the oxidative stress biomarkers of the heart tissue of the animals were studied. Reduction in SOD, GSH, catalase and increase in MDA shows an increased oxidative stress. Oxidative stress occurs due to excess production of free radicals. In the absence of antioxidants, oxidative stress results and this can lead to different pathological conditions including cardiovascular diseases.[18] Superoxide dismutase, glutathione and catalase serve as protection against free radicals. Treatment with extracts led to increase in superoxide dismutase (SOD), glutathione (GSH) and catalase, showing that both extracts alone and the combined extracts had protective effect against oxidative stress in the heart tissue of the rats.

CONCLUSION

The use of natural products from plants in the treatment of different diseases is on the increase; this is due to their rich phytochemical contents. Treatment with 100mg/kg body weight of ethanol leaf extract of *A. muricata*, *A. heterophyllus*, 100 and 200 mg/kg body weight of the combined extracts reduced hyperlipidaemia associated with consumption of high fat diet. The extracts also reduced the effect of oxidative stress on the heart tissues of the animals and reduced the systolic blood pressure of the hypertensive rats.

REFERENCES

1. Florence NT, Benoit MZ, Jonas K, Alexandra T, Désiré DDP, Pierre K, Théophile D. Antidiabetic and antioxidant effects of Annona muricata (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. Journal of Ethnopharmacology, 2014; 151(2): 784-790.
2. Kossouoh C, Moudachirou M, Adjakidje V, Chalchat JC, Figuérédo G. Essential oil chemical composition of Annona muricata L. leaves from Benin. Journal of Essential Oil Research, 2007; 19(4): 307-309.
3. Sawant TP, Dongre RS. Bio-chemical compositional analysis of Annona muricata: a miracle fruit's review. International Journal of Univers Pharmacy and Bioscience, 2014; 3: 82-104.
4. Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M., and Kadir, H. A.Annona muricata (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. International Journal of Molecular Sciences, 2015; 16(7): 15625-15658.
5. Shibula K, Velavan S. Determination of Phytocomponents in Methanolic Extract of Annona muricata Leaf Using GC-MS Technique. International Journal of Pharmacognosy and Phytochemistry Research, 2015; 7(6): 1251-1255.
6. Swami SB, Thakor NJ, Haldankar PM, Kalse SB. Jackfruit and its many functional components as related to human health: a review. Comprehensive Reviews in Food Science and Food Safety, 2012; 11(6): 565-576.
7. Madruga MS, de Albuquerque FSM, Silva IRA, do Amaral DS, Magnani M, Neto VQ. Chemical, morphological and functional properties of Brazilian jackfruit (Artocarpus heterophyllus L.) seeds starch. Food chemistry, 2014; 143: 440-445.

8. Sindhu SN, Nithyakala CM, Ivy GN, Nazia S, Somashekharaiah BV. Isolation and determination of nutritional and antinutritional compounds from the seeds of selected plant species. Journal of Chemical and Pharmaceutical Research, 2012; 4(7): 3529-3534.
9. Prakash O, Kumar R, Chandra D, Kumar A, Kumar P. Effect of Artocarpus heterophyllus Lam. (Jackfruit) on Indomethacin-Induced ulcer model in albino rats. Der Pharmacia Lettre, 2015; 7(1): 81-85.
10. Prakash O, Kumar JA, Gupta, R. Evaluation of anticonvulsant activity of Artocarpus heterophyllus Lam. leaves (Jackfruit) in mice. Der Pharmacia Lettre, 2013; 5(1): 217-220.
11. Omar HS, El-Beshbishy HA, Moussa Z, Taha KF, Singab ANB. Antioxidant activity of Artocarpus heterophyllus Lam. (Jack Fruit) leaf extracts: remarkable attenuations of hyperglycemia and hyperlipidemia in streptozotocin-diabetic rats. The Scientific World Journal, 2011; 11: 788-800.
12. Niroumand S, Khajedaluee M, Khadem-Rezaiyan M, Abrishami M, Juya M, Khodaee G, Dadgarmoghaddam M. Atherogenic index of plasma (AIP): A marker of cardiovascular disease. Medical Journal of the Islamic Republic of Iran, 2015; 29: 240.
13. Mopuri R, Ganjayi M, Banavathy KS, Parim BN, Meriga B. Evaluation of anti-obesity activities of ethanolic extract of Terminalia paniculata bark on high fat diet-induced obese rats. BMC Complementary and Alternative Medicine, 2015; 15(1): 76.
14. Delacroix S, Chokka RC, Worthley SG. Hypertension: Pathophysiology and treatment. Journal of Neurology and Neurophysiology, 2014; 5(250): 2.
15. Olafiranye O, Zizi F, Brimah P, Jean-louis G, Makaryus AN, McFarlane S, Ogedegbe G. Management of hypertension among patients with coronary heart disease. International Journal of hypertension, 2011.
16. Duquette D, Theisen V, Beene-Harris R, Bach J, Kardia S, Wang C. PEER REVIEWED: Blood Pressure Sunday: Introducing Genomics to the Community Through Family History. Preventing chronic disease, 2005; 2: 2.
17. Chackrewarthy S, Thabrew MI. Hypoglycaemic and hypolipidaemic effects of an ethylacetate fraction of Artocarpus heterophyllus leaves. In Glucose Tolerance. Intech. 2012.
18. Ullah AA, Khan AA, Khan I. Diabetes mellitus and oxidative stress—A concise review. Saudi Pharmaceutical Journal, 2016; 24: 547–553.