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Evaluation of the Effects of Treatment of Normal Albino Wistar Rats with Ethanol leaves extract of *Mangifera indica* and *Gongronema latifolium* on the Lipid Profile.

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ABSTRACT
The Effects of ethanolic leaves extracts of Mangifera indica and Gongronema latifolium on the lipid profile (TC, TG, HDL, LDL and VLDL) of normal albino wistar rats was evaluated. Sixteen animals (185-221g) were randomly assigned four groups of four rats each. Groups 1 and 3 were treated with 200mg/kg ethanol leave extracts of Mangifera indica and Gongronema latifolium resepectively. Group 2 was treated with 200mg/kg leave extracts of Mangifera indica and Gongronema latifolium at 50:50 dosage ratio. Group 4 was not treated with extract and served as control. All animals were allowed free access to commercial rats mash and water throughout 3 weeks treatment period. At the end of the 21 days treatment, the results of serum total cholesterol (TC) showed a significant decrease (P< 0.005) in all treatment groups when compared to the control. Serum triaglyceride (TG) level revealed a significant decrease (P<0.05) in groups 1 and 2 and no significant difference (P> 0.05) was seen in group 3 when compared to the control. There was a significant decrease (P< 0.05) in serum HDL in all treatment groups when compared to the control. Serum VLDL demonstrated a significant decrease (P< 0.05) in group 1 and 2 and a significant increase in group 3 when compared to the control. No significant difference (P> 0.05) was observed in serum LDL in groups 2 and 3 and no significant difference (P > 0.05) in group 1 when compared to the control. The implications of these results are discussed.
Keywords: *Mangifera indica, Gongronema latifolium, Lipid profile, Albino Wistar rats.*

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I. INTRODUCTION

Lipids are any of a diverse group of organic compounds including fats, oil, hormones and certain components of membranes that are grouped together because they do not interact appreciably with water. They are micro-biomolecules that are soluble in non-polar solvents (Zhang *et al.,* 2008). The function of lipids includes storing energy, signaling and acting as structural components of cell membrane (Nelson and Cox, 2000). Lipids are used in the cosmetic and food industries as well as in nanotechnology (Mashagri *et al.,* 2013). The occurrence of many cardiovascular diseases is becoming a threat to public health in the society, and this is culminated by hyperlipidemia. Meanwhile, some plants have medicinal uses as they are used for the treatment of some diseases (Arumegan *et al.,* 2009). Plants basically have been an essential part of human society since the beginning of civilization as it plays an important role in the absorption of carbon(iv)oxide and release oxygen from their leaves which humans and other organisms need for their survival. These medicinal plants also posses significant clinical values in the treatment of microbial strains and are considered as rich resources of ingredients which can be used in the drug development (Hammer *et al.,* 2001).

Furthermore, some of the plants with this medicinal efficacy are *Mangifera indica* and *Gongronema latifolium. Mangifera indica* commonly known as mango, is a specie of flowering plant in the sumac and poison ivy family *Anacardiaceae*. It is a large tree fruit capable of growing to a height and crown width of about 30 metre (100ft) and truck circumference of 3.7 metres (12ft) (Kuhn *et al.,* 2017). *Mangifera indica* is known for its immunology, antioxidative, anti-ageing, anticancer, antidiabetic, hepatoprotective, analgesics effects (Smith and Harbone, 2005). *Gongronema latifolium* is locally known as Utazi, belongs to the family *Asclepiadaceae*. It is a green climbing plant with a bitter petiolate leaves, usually grown from stems or seedling of the plant (Okafor *et al.,* 1996). It is one of the important medicinal, economic and indigenous plant found in the humid region of South Eastern and South Southern Nigeria (Kuhn *et al.,* 2017). It is also basically used as spice in foods as well as used traditionally in the treatment of diabetes, hypertension, cardiovascular diseases as well gastro-intestinal problems (Ugochukwu *et al.,* 2003). Lipid and lipoprotein abnormalities play a role in the

pathogenesis and progression of atherosclerosis and cardiovascular diseases (Chrysohoov *et al.,* 2004). Therefore, this work seeks to explore and confirm the effect of *Mangifera indica* and *Gongronema latifolium* leaves extracts on lipid profile of normal albino wistar rats.

II. MATERIALS AND METHODS

Collection of the Plant Samples

The fresh leaves of *Mangifera indica* and *Gongronema latifolium* were collected at different locations within Ikot Ekpene and Abak Local Government Areas, both in Akwa Ibom State, Nigeria. The two leaves were authenticated by a Taxonomist in the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Preparation of the Plant Samples

The two plant leaves were plucked from their stems washed with distilled water to remove dirt, sliced separately with knife into tiny pieces and dried separately at room temperature for 3 days. The dried leaves were later pounded separately using clean, dry mortar and pestle and 500g each of the samples were soaked in 100ml of 70% ethanol for 72 hours at room temperature. The macerated leave extracts were differently filtered using Whatman No. 1 filter paper by means of a funnel. The filtrates were separately concentrated at 40^0C-50^0C in a water bath for 3 consecutive days, after which slurry form of the extracts obtained and preserved in a refrigerator at 4^0C for further use.

Experimental Design, Grouping and Treatment of the Animals

Sixteen healthy adult male albino wistar rats weighing 185-220g were obtained from the disease free stock of the animal house of the University of Calabar, Cross River State, Nigeria. The animals were housed in a cage with four sizeable compartments of wooden bottom and wire mesh top, randomly assigned four animals per four groups. The rats were maintained under standard conditions of temperature and natural light-dark cycle for 7 days acclimatization in the animal house, Akwa Ibom State Polytechnic, Ikot Osurua. Groups 1 and 3 animals were treated with 200mg/kg of *Mangifera indica* and *Gongronema latifolium* leave extracts respectively, while groups 2 was treated with 200mg/kg combined extracts of *Mangifera indica* and *Gongronema latifolium* leaves at 50:50 dosage ratio. Animals in the remaining group were not treated with the leave extracts and served as normal control. Treatment was done orally daily for three weeks. The animals were all allow free access to commercial rat mash and water throughout the duration of extracts treatment. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from the cages daily.

Collection of Blood Sample and Preservation of Serum

At the end of 21 days treatment period, the rats were made to undergo overnight fast and were anaesthetized under chloroform vapour and were sacrificed by dissecting medioventrically. Blood was collected via cardiac puncture by means of syringe and needle into a sterile EDTA sample bottles and then centrifuged at 4, 000rpm for 25minutes to separate serum from the blood cells. The serum was preserved at 4^0C in a refrigerator till when needed for lipid profile assay.

LIPID PROFILE ASSAY

Determination of High-Density Lipoprotein (HDL) (Friedewald *et al.,* 2003)

About 100µl of sample was pipetted into centrifuge tubes and 100µl of HDL precipitant added, the content was mixed and allowed to stand for 10 minutes at room temperature, then centrifuged for 10 minutes at 400rpm. The clear supernatant was collected within 24 hours into sterile plain-tubes and labeled appropriately. About 50µl of distilled water, standard cholesterol and supernatant were taken into plain tubes, labeled blank, standard and sample respectively. The contents were mixed and incubated at room temperature for 10 minutes. The absorbances of the contents in the tubes were read at 546nm against reagent black within 60 minutes. The concentration of HDL was calculated thus;

$$\text{Concentration of HDL (mmol/l)} = \frac{\text{Ab sample}}{\text{Ab standard}} \times \frac{\text{concentration of standard}}{1}$$

Where Ab = Absorbance.

Determination of Low-Density Lipoprotein (LDL) (Friedewald *et al.,* 2003)

Low Density Lipoprotein (LDL) was calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship;

$$\text{Concentration of LDL (mmol/l)} = TC - \frac{TG}{2.2} - HDL$$

Where
TC = Total Cholesterol
TG = Triglyceride
HDL = High Density Lipoprotein

Determination of Triglyceride

Sterile plain tubes were labeled as blank, standard and sample accordingly. Sample tubes were arranged serially according to the number of samples. Exactly 10µl of standard and sample were pipetted into their respective tubes using micro pipettes, while the blank tube had nothing at this point. One vial of enzyme reagent R1b was reconstituted using 15ml of R1a buffer. Thereafter, 1000µl (1ml) of this solution was added to all the tubes including the blank, mixed and incubated at room temperature for 10 minutes. At the end of 10 minutes, the absorbance (Ab) of all the contents in the tubes were read at 546nm and recorded. Concentration of Triglyceride (TG) was calculated thus;

$$\text{Concentration of TG (mmol/l)} = \frac{\text{Ab sample}}{\text{Ab standard}} \times \frac{\text{concentration of standard}}{1}$$

Determination of Total Cholesterol (TC)

Serum total cholesterol was assayed by the method by Sharul *et al.* (2013). Sterile plain tubes labeled according to the number of samples were arranged serially. Exactly 10µl of sample, distilled water and standard were pipetted into tubes labeled sample, blank and standard respectively. Also, 1000µl (1ml) of cholesterol reagent (R_1) was added across board at room temperature. The tubes were shaken to get the contents mixed and incubated for 10 minutes at room temperature. The spectrophotometer was adjusted to a wavelength of 546nm and standardized with the reagent blank to read a zero absorbance. the absorbance of all the tubes were measured and recorded within 30 minutes. The concentration of TC was calculated thus;

$$\text{Concentration of TC (mmol/l)} = \frac{\text{Ab sample}}{\text{Ab standard}} \times \frac{\text{concentration of standard}}{1}$$

Determination of Very Low-Density Lipoprotein (VLDL)

The concentration of VLDL cholesterol in the samples was determined by calculation as follows;

$$\text{Concentration of VLDL (mmol/l)} = \frac{\text{TG}}{2.2}$$

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM). The data obtained from the experiment were subjected to one-way analysis of variance (ANOVA). Significant differences were obtained at P. < 0.05. This was estimated using statistical package for social sciences (SPSS) version 23.

Table 1: Mean Serum Lipid Profile in Albino Wistar Rats treated with Ethanol leaves Extracts of *M. indica* and *G. latifolium* plants

Parameters	Control	Group 1	Group 2	Group 3
TC (mmol/l)	1.73± 0.05	1.43± 0.05	1.23± 0.03	1.38± 0.06
TG (mmol/l)	0.88± 0.03	0.63± 0.02	0.60± 0.04	1.10± 0.14
HDL (mmol/l)	1.80± 0.04	0.50± 0.09	1.18± 0.05	1.08± 0.08
LDL (mmol/l)	0.47± 0.09	0.64± 0.05	0.22± 0.07	0.29± 0.09
VLDL (mmol/l)	0.40± 0.01	0.29± 0.02	0.27± 0.02	0.50± 0.06

Values were expressed as mean ± SEM (N = 4). Significant difference was accepted at P < 0.05.

III. DISCUSSION

Plants and plant products have been used over the years for medicinal purposes. It has been estimated that more than 25% of prescribed pharmaceuticals contain plant derived ingredients, yet only a small amount of the plants in the world have been evaluated for potential pharmaceutical use (Cobiac, 2006). Lipid and lipoprotein abnormalities play a major role in the development and progression of coronary artery diseases (CVD). Among the cardiovascular complications, atherosclerosis is responsible for approximately 50% of death in western countries (Rehrab *et al.,* 2007). Cardiovascular disease is characterized by the elevation of serum

triglyceride (TG), total cholesterol (TC) and low-density lipoprotein (LDL) with a decrease in high-density lipoprotein (HDL) with a decrease in high-density lipoprotein (HDL) values (Bruckner, 2008). However, negative alterations in these lipid profile fraction provides useful information concerning the status of lipid metabolism as well as predisposition to atherosclerosis and its complications (Singh *et al.,* 2012). Under this condition, lipids and other related substances accumulates on the arterial wall, forming plague, which occlude the vascular lumen and obstruct the flow of blood to vital organs such as heart, brain, liver etc.

Meanwhile this work sought to evaluate the effect of ethanol leave extracts of *Mangifera indica* and *Gongronema latifolium* on the lipid profile of normal albino Wistar rats. The results revealed a significant decrease in the mean serum total cholesterol (TC) levels in all the treated groups when compared to the control. Significant decrease was also recorded in serum TC in group 2 when compared to group 1, but no significant difference was observed in group 3 when compared to group 1. Significant increase in serum TC level in group 3 was exhibited when compared to group 2, though the values where within the normal ranges for human (3.1-6.8mmol/l). These results implied that the two leaves extracts were able to regulate serum TC levels in the experimental groups of animals within their normal range. This signified that the liver was able to metabolize the extracts well and was in normal physiologic condition. However, increase of serum total cholesterol above its normal range is a predisposing factor to cardiovascular diseases (CVD), stroke, peripheral diseases etc. Invariably, decrease in serum TC level below the normal range can result in low birth weight in pregnant woman, cancer and depression (Fahy *et al.*, 2009). The results of serum TC levels obtained in this work was in line with the results of findings of Cheek (1991) who reported significant decrease in serum TC in albino wistar rats treated with ethanol leaf extract of *Mangifera indica* when compared to the control (normal).

There was significant decrease in the mean Triglyceride (TG) levels in groups 1 and 2 when compared to the control. But, significant increase was recorded in serum TG level in group 3 when compared to the control, while no significant difference was observed in group 1 when compared to the group 2. However, serum TG levels in all treatment groups fell within the standard range (0.4 -2.0mmol/l) for human. An increase in serum TG levels above the normal range in human contributes to the hardening of the arteries or thickening of the artery walls, stroke, heart attack, acute inflammation of the pancreas. But, a decrease in serum TG levels below normal range is associated with the risk of death in persons with stroke and in women who suffered heart failure (Sirton, 2006). The results of serum TG was in agreement with the work by Benayoun *et al.* (2007), who reported a significant increase in serum TG in rats treated with 200mg/kg of *Gongronema latifolium* aqueous leaf extract.

Mean serum high-density lipoprotein (HDL) in groups 1, 2 and 3 were significantly decreased when compared to the control. Significant decrease was also recorded in group 1 when compared to groups 2 and 3, but no significant difference was observed in group 2 when compared to group 3. However, the mean serum HDL when compared to the normal range (1.0 - 1.6mmol./l) for male and (1.3 - 1.6mmol/l) for female was below the normal range in group 1 animals, but within the normal range in groups 2 and 3, for both male and female. But, there was an increase in the control group of animals above the normal range for both male and female. This demonstrated that the extract when combined worked antagonistically to produce a better effect. Also, single treatment with *G. latifolium* also had a better effect by regulating serum HDL level to fall within the normal range. But single administration with *M. indica* leave extract reduce the mean serum HDL level as seen in group 1 animals which was below the normal range. This study was in accordance with the investigation by Benayoun *et al.* (2007) who reported a significant increase in serum HDL in rats treated with 200mg/kg of *G. latifolium* leaf extract. HDL is a good cholesterol and plays a cardioprotective role. It contributes to the reverse cholesterol transport and removal of cholesterol from the peripheral tissues. Furthermore, HDL plays an antioxidant role and prevent LDL from getting oxidized (Satyananyara and Chakrapan, 2014). This indicates that atherosclerosis and other complications like heart attack could be reduced. Hence, an increase HDL level if considered advantageous as it is a good cholesterol, while low HDL level can result in atherosclerosis.

Furthermore, mean serum low-density lipoprotein (LDL) recorded significant increase in Group 1 when compared to groups 2 and 3 respectively while no significant difference was recorded in all treatment groups when compared to group 3. Meanwhile, all the mean serum LDL in the treatment groups in the control, fell below the normal range (2.6-3.3mmol/l) for human. This observation was in line with a report by Corlett *et al.* (2002) who reported significant effect of aqueous extracts of *M. indica* and *Kola acuminata* on the lipid profile of 16 adult albino wistar rats. Their reports revealed a significant effect of the extracts on serum HDL, TC, TG and VLDL, with a non significant effect on serum LDL. However, the result of this work showed that the leave extracts has no effect on serum LDL. A high serum LDL correlates with high incidence of coronary heart disease, particularly, atherosclerosis. Mean serum very low-density lipoprotein (VLDL) level in group 2 was significantly increased when compared to treatment group 1 and 2 respectively and when compared to the control. But, no significant difference was recorded in group 1 when compared to groups 2. The mean serum VLDL levels in all the treatment groups and the control fell within the normal range of 0.1-1.7mmol/l for human. This result was also in accordance with the work by Smith and Harbone (2005) who reported a

significant effect of ethanol leaf extract of *G. latifolium* (200mg/kg) on serum VLDL in albino wistar rats. Therefore, regulation of mean serum lipid profile in this study within their normal ranges was demonstrated by the hypolipidemic potentials of the two leave extracts used in this work.

IV. CONCLUSION

The regulation of lipid metabolism by lipid metabolizing agents is of great importance in the wellbeing of an individual with regards to cardiovascular diseases (CVDs). *Mangifera indica* and *Gongronema latifolium* leaves extracts have displayed hypolipidemic properties by regulating lipid profile within the normal ranges in the albino wistar rats under study. Therefore, the two leaves extracts could be used to manufacture drugs to arrest cardiovascular diseases which are basically caused by elevated serum LDL, TG, TC and VLDL, with depressed serum HDL.

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