

**EVALUATION OF THE EFFECT OF ADMINISTRATION OF
ETHANOL EXTRACTS OF *ANDROGRAPHIS PANICULATA* LEAVE
AND *ZINGIBER OFFICINALE* RHIZOIDS ON SERUM LIPID
PROFILE IN NORMAL WISTAR RATS.**

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ABSTRACT

The effect of ethanol extracts of *Andrographis paniculata* leaves and *Zingiber officinale* rhizoids on serum lipid profile on albino wistar rats was determined. Twenty-five (25) animals weighing 113-233g were randomly assigned five groups of five animals each and one of the groups was randomly chosen to serve as control. Group 1, 2, 3 and 4 (treated groups) were respectively treated with 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg of combined extracts of the two plants at 50:50 dosage ratio for 3 weeks. Both control and treatment groups of animals were fed with commercial rat mash and with distilled water. The result revealed that there was no significant difference in the mean serum Triglycerol (TG), Total Cholesterol (TC), Low Density

Lipoprotein (LDL and Very Low Density Lipoprotein (VLDL) in the treatment groups respectively when compared with the control ($p < 0.05$). but a significant increase was recorded in the mean serum High Density Lipoprotein (HDL) in the treatment group 2 when compared with group 1 ($p > 0.05$). The implications of this result was discussed.

KEYWORDS: *Andrographis paniculata*, *Zingiber officinale*, Albino wistar rats, Lipid Profile, Ethanol Extract.

INTRODUCTION

The use of medicinal plant is a core component of primary health care level due to availability, acceptability, affordability and compatibility. Depending on these, medicinal

plants varies from country to country, it is estimated that about 75-78% of people in the developing countries and 'about 25% of people in the developed countries depend either directly or indirectly on medicinal plants for the first line of treatment (Duke, 2007). Medicinal plants play a vital role in traditional health care systems for curing many diseases. Medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Kavishankor *et al.*, 2011). Medicinal plants include various types of plant used in herbalism and some of these plants have a medicinal potency. These medicinal plants are those considered as rich resources of ingredients which can be used in drug development and synthesis (Rasool, 2012). Besides that these plants play a critical role in the development of human cultures around the whole world. It plays a contract role not only as traditional medicines but also as trade commodities, meeting the demand of distant markets. Ironically, India has a very small share (1.6%) of this ever growing global market (Schippman *et al.*, 2002). Medicinal plants with antioxidant activities have also been shown to be useful for the prevention of Artherosclerosis and cardiovascular disease by reducing lipid peroxidation (HeIdarianet *et al.*, 2013).

Medicinal plants however, have a long history of use for the benefit of mankind. Example of such plants are *Andrographis paniculata* and *Zingiber officinale*. Thus, *Andrographis paniculata* is an annual herb (Kuppusamy and Murugan, 2008) possessing immense therapeutic uses (mostly used parts are roots, leaves and aerial part of mature twig). It is also reported to be a perennial shrub (Hancharnlerd, 1994). It is native to India and Sri Lanka. This plant is traditionally used for the treatment of common cold, diarrhea, fever due to severe infection, jaundice and as a health tonic for the liver and cardiovascular health and as an antioxidant (Joy *et al.*, 1998). It is also used for the treatment of cough with thick sputum, carbuncle, and snake bite. *Andrographis paniculata* has been reported to exhibit various mode of biological activities *in vivo* as well as *in vitro* viz, antibacterial (Singha *et al.*, 2003), anti- inflammatory (Wen *et al.*, 2010), anti HIV (Human Immune Deficiency Virus) (Chao *et al.*, 2010). All parts of these plants are used to extract the active phytochemical, but the compositions of phyto constituents widely differ from one part to another and with place, season and time of harvest. *Zingiber officinale* rhizoid is a perennial herb widely cultivated in the tropics and which occasionally naturalizes it mostly spreads vegetatively, since many cultivars seldom flower or are sterile (Sutarno *et al.*, 1999). It belongs to the family *Zingiberaceae*, in the order zingiberales of monocotyledons, which is composed of 50

genera and around 1500 species of perennial tropical herbs. It contains a wide variety of biologically active, nonnutritive compounds known as phytochemicals (Sheetal *et al.*, 2009). For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicines for the treatment of catarrh, rheumatism, nervous disease gingivitis, toothache, asthma, stroke, constipation and diabetes (Tapsell *et al.*, 2006).

MATERIALS AND METHODS

Collection of the Plant samples

The fresh leaves of *Andrographis paniculata* were collected in June, 2019 from the botanical garden in Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene Local Government Area of Akwa Ibom State. *Z. officinale* rhizoid was bought from Oto market from the above Local Government Area both in Akwa Ibom State. The two samples were identified and authenticated by a Taxonomist in the Department of Botany, University of Uyo, Akwa Ibom State, Nigeria and were later taken to the Biochemistry Laboratory in Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene for preparation and use in the research.

Preparation of the Plant Samples

Andrographis paniculata leaves were plucked from the stem and *Zingiber officinale* rhizoids were scrubbed with the aid of a knife to remove the bark. Both samples were separately washed in a clean water to remove debris, shred and were air-dried for 48 hours under shed. The dried samples were ground separately to powder form using an electric blender and were stored in air-tight containers and labeled correctly. About 170kg of powdered *Andrographis peniculata* was macerated in 3500ml of 70% ethanol and 185kg of powdered *Zingiber officinale* was macerated in 2500ml of 70% ethanol for 72 hours respectively at room temperature (25°C). The mixtures were then filtered separately using Whatman's No 1 filter paper over a funnel. The filtrates were separately concentrated through water bath at 40-50°C for three consecutive days to get the slurry form of the extracts. They were preserved in a refrigerator at 4°C for further use.

Experimental Design, Grouping and Treatment of the Animal

Twenty-five (25) albino wistar rats weighing (113-233g) of male sex were obtained from the disease free stock of the animal house, Biochemistry Unit, Department of Science Technology, Akwa Ibom State Polytechnic, Ikot Osurua. The animals were randomly

assigned five (5) groups of five rats each. The animals were housed in wooden cages under standard conditions for acclimatization for one week in the experimental animal house before the commencement of the experiment. Each group was weighed to obtain the mean body weight. Group 1 received a combined extract of *Andrographis paniculata* (50mg/kg) and *Zingiber officinale* (50mg/kg). Group 2 received a combined extract of *Anarograpnis paniculata* (100mg/kg) and *Zingiber officinale* (100mg/kg). Group 3 received a combined extracts of *Andrographis paniculata* (150mg) and *Zingiber officinale* (150mg/kg). Group 4 received a combined extracts of *Andrographis paniculata* (200mg/kg) and *Zingiber officinale* (200mg/kg). Group 5 was the normal control without extract administration but were fed with normal rat mash and distilled water for three weeks. The experimental groups were equally fed with rat mash and distilled water throughout the period of treatment. The extracts were administered daily through oral route for three weeks (21days). Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily.

Collection of Blood Sample, and Preservation of Serum

After 21 days (3 weeks) of extracts administration and feeding, the animals were subjected to overnight fast, then they were anaesthetized with chloroform vapour and were sacrificed by dissecting medioventrically and the blood sample collected through cardiac puncture by means of syringe and needle into well labeled anticoagulant (EDTA) bottles and gently shaken and allowed to stand for 1 hour after which they were centrifuged at 4,000rpm for 10minutes to separate serum from the blood cells. The serum obtained was used for the determination of Total Cholesterol (TC), tricylglycerols (TG), High-Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very-Low Density Lipoprotein (VLDL) which constitute the lipid profile.

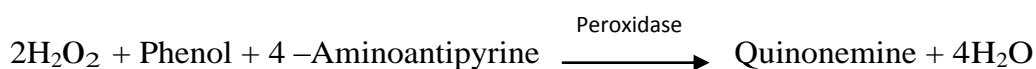
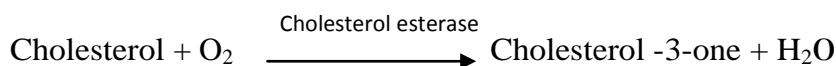
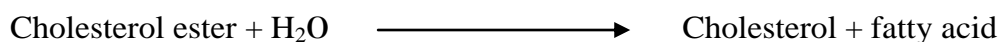
Determination of Total Cholesterol (Tc)

Determination was based on method of Abell *et al* (1993).

Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Cholesterol esterase



To 3 samples tubes labeled reagent blank, standard, and sample, 10µL of distilled water, standard and sample were respectively added. Then to each tube, 100 µL of the reagent was added, mixed and incubated for 10 minutes at 20 - 25°C. the absorbance of the sample (A sample) and standard (A standard) were read against the reagent blank within 60 minutes with spectrophotometer at wavelength of 500nm.

Calculation

Total cholesterol in sample (mmol/l)

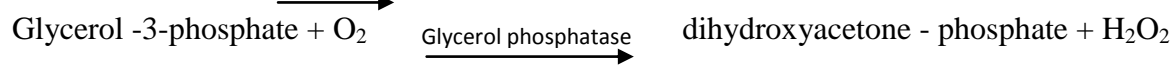
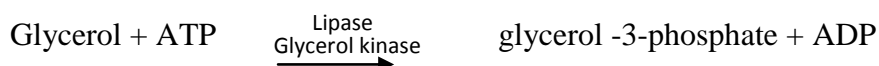
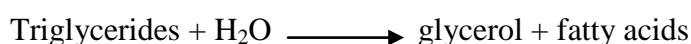
$$= \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times \frac{\text{concentration of standard}}{1}$$

Determination of Triacylglycerol (TG)

The triacylglycerol has been determined by the method described by Abell *et al* (1993).

Principle

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.



About 10µL serum sample was pipetted into clean test tubes and labeled sample. Then to another bottle labeled standard, 10µL of the standard was pipetted and 100µL of the reagent was pipetted into each of the test tubes. It was mixed and incubated for 10 minutes at 20-25°C. Then the absorbance of the sample (A sample) and that of the standard (A standard) were measured against the reagent blank within 60 minutes at a wavelength of 546nm using spectrophotometer.

Calculation

Triglycerol (TG) concentration

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{concentration of standard}}{1}$$

Determination of High-Density Lipoprotein (HDL)

HDL cholesterol was determined by the method described by Abell *et al* (1993).

Principle

Low-density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (High density Lipoprotein) fraction, which remains in the supernatant is determined.

Precipitation: To 2 sample tubes labeled sample and standard, 100μL of sample and 100μL of standard was respectively added.

Then to each tube, 250μL of the diluted precipitant was added, mixed and allowed to stand for 10 minutes at room temperature. It was then centrifuged for 10 minutes at 4000rpm. The supernatant obtained from centrifugation was separated into a test tube.

Then 100μL of distilled water was measured into a test tube labeled reagent blank, and 100μL of the supernatant was measured into another test tube labeled standard. Finally, 1000μL of cholesterol reagent was added into each of the test tubes. It was mixed and incubated for 10 minutes at 37°C after which the absorbance of the sample and standard were measured against reagent blank for 60 minutes at 500nm.

Calculation

$$\begin{aligned} &\text{Concentration of high density lipoprotein (mmol/l)} \\ &= \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times \frac{\text{concentration of standard}}{1} \end{aligned}$$

Determination of Very Low Density Lipoprotein (VLDL),

This was also done by calculation using the formula

$$\text{VLDL (mmol/l)} = \frac{\text{Triglycerides}}{2.2}$$

Statistical Analysis

The data obtained from the test were subjected to one-way analysis of variance (ANOVA) using SPSS. Significant differences were obtained at $P < 0.05$ by Boniferroni multiple range test. The results were expressed as mean \pm standard error of mean (SEM). This was estimated using statistical package for social science (SPSS) version 20.

Table 1: Mean Serum Lipid Profile of Albino Wistar Rats Treated with Combined Ethanol Extracts of *A. paniculata* Leaves and *Z. officinale*.

Groups/Treatment	TC mmol/l	TG mmol/l	HDL mmol/l	LDL mmol/l	VLDL mmol/l
Group 1 (100mg/kg)	1.20 \pm 0.09	0.52 \pm 0.10	0.44 \pm 0.03	0.52 \pm 0.08	0.24 \pm 0.05
Group 2 (200mg/kg)	2.06 \pm 0.64	0.84 \pm 0.37	0.76 \pm 0.20	0.93 \pm 0.27	0.36 \pm 0.18
Group 3 (300mg/kg)	1.27 \pm 0.10	0.39 \pm 0.10	0.51 \pm 0.06	0.61 \pm 0.09	0.16 \pm 0.06
Group 4 (400mg/kg)	1.18 \pm 0.11	0.32 \pm 0.02	0.54 \pm 0.04	0.51 \pm 0.13	0.15 \pm 0.15
Group 5 control (mg/dl)	1.52 \pm 0.23	0.61 \pm 0.24	0.56 \pm 0.04	0.68 \pm 0.26	0.28 \pm 0.11

Results are presented as a mean \pm S. E. M

DISCUSSION

Lipid and lipoprotein abnormalities play a role in the development and progression of coronary artery disease. Among the cardiovascular complications, atherosclerosis is responsible for approximately 50% of death in western countries (Rehrah *et al.*, 2007). Cardiovascular disease is characterized by the elevation of serum TC, TG and LDL with a decrease in HDL values (Brucker, 2007). Medicinal plants are integral part of human life to combat the sufferings from the dawn of civilization. However, various studies have shown the benefit of plant extracts on the cardiovascular disease risk development and the reduction of the disease itself. *Andrographis paniculata* leaf and *Zingiber officinale* rhizoids are some of those plants that are being used in Nigeria for the treatment of cardiovascular diseases such as cold, fever, diabetes, stroke due to their active components; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This research work investigated the effects of ethanolic extracts of *Andrographis paniculata* leaf and *Zingiber officinale* rhizoids on lipid profile in albino wistar rats. Statistically, serum levels of Total Cholesterol (TC), Triacylglycerol (TG), Low-Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Cholesterol and Very Low Density Lipoprotein (VLDL) were evaluated in albino rats treated with combined doses of *Andrographis panitulata* leaf and *Zingiber officinale* rhizoids extract.

Cholesterol is an important biomolecule involved in array of cellular functions such as maintenance of membrane fluidity, production of hormones, production of vitamin D on the surface of the skin etc. (Daniel *et al.*, 2009). However, for effective functionality, Total Cholesterol (TC) must fall within the normal range of 3.8- 6.2 mmol. Alteration of total cholesterol above this physiological range plays an important role in the initiation and aggravation of atherosclerosis known to have positive correlation with cardiovascular diseases and other associated clinical disorders (Hsu, 2003). On the other hand, changes in total cholesterol below the normal range is believed to barricade the stated functions. The results of the co- administration of *Andrographis paniculata* leaf and *Zingiber officinale* revealed no significant alteration in TC levels in all the treated animals compared with the control. This result is in line with the report of Eugene and Manavalan (2011), that administration of ethanol extract of *Andrographis paniculata* at 100, 200 and 300mg/kg did not alter significantly the levels of total cholesterol in albino rats. This signifies that the lipid (cholesterol) metabolizing machinery of the molecules were not affected.

High-density Lipoprotein (HDL) is a lipid transporter that convey cholesterol and cholesteryl esters from the peripheral tissues and cells to the liver for metabolism into bile acids. HDL-C plays a prominent role in the reduction of inflammation, protection against oxidation of LDL, interfere positively with blood clotting and decrease plaque formation within the walls of the arteries. However, for this cardioprotective functions to be achieved, HDL fraction must be within the physiological range of 1.0-1.2mmol/l. The results of the present study revealed no significant change in HDL in the treated groups except a significant increase in group 2 treated with 200mg/kg extracts when compared with group 1 treated with 100mg/kg. The significant increase was below the normal range of 1.0- 1.2mmol/l. According to Ademuyiwa *et al* (2005), increase HDL exerts a protective effect by decreasing the rate of entry of cholesterol into the cell via LDL and increasing or promoting reverse cholesterol transport and subsequently esterifying the lipid using lecithin cholesterol acyltransferase and deliver to the liver and other steroidogenic organs for bile synthesis. Furthermore, HDL plays an antioxidant role due to the enzyme paraoxonase activity and protect LDL from getting oxidized (Satyanayara and Chakrapani, 2014). This indicates that atherosclerosis and other complications like heart attack could be reduced. Low-Density Lipoprotein (LDL) and Very Low- Density Lipoprotein Cholesterol (VLDL) are considered as bad cholesterol

and they aggravate cardiovascular disorder. Studies have shown that high LDL above the normal range of 1.5- 3.5mmol/l and VLDL above 0.1-1.7mmol/l are the major cardiovascular risk factor (Philip, 2007). The molecules pose this risk when they invade the endothelium and become oxidized resulting to occultation of the arterial walls with fatty materials. The result of LDL and VLDL in the present study revealed no significant alterations in the treated animals compared to the control. The result is similar to the report of Dhanik *et al* (2017) that serum LDL and VLDL of albino rats treated with 250 and 500mg/kg of *Zingiber officiale* did not cause any significant change.

Triacylglycerol (TG) is a major component of VLDL and chylomicrons that play an important role in the metabolism as energy source as well as functioning as membrane component. However, despite the function of TG, it must not exceed the physiological range of 0.4-2.0mmol/l. According to Davidson *et al* (2009), high levels of TG in the blood have linked to arteriosclerosis and by extension, heart diseases. The result of TG in the present study indicates no significant alteration statistically in all the treated groups compared with the control. The results obtained is similar to the report of Mathivanana and Edwin (2012); Zang and Tan (2000) that serum TC, TG, LDL and VLDL were not significantly altered rats treated with 200,400 and 600mg/kg of *Andrographis paniculata*. Furthermore, Dhanik *et al* (2017) reported no significant changes in TC, TG, LDL and VLDL. It is observed that the result of TG was within the normal range. The insignificant results obtained are as an indication that the metabolizing machinery of lipids were not affected by the extracts. This probably may be due to the fact that the oxidation of the fraction of lipid profile were counter balanced by the phytochemicals preferably the antioxidant molecules (Flavonoids, Phenols and Saponins). The extracts though did not significantly alter TC, LDL and VLDL, the downward trend observed in all the treated groups cannot be overlooked since low LDL-C correlate with low incidence of coronary heart diseases.

According to Lipid Research Clinic Programme, 1984 an increase of 1% cholesterol is reported to have resulted in 3% increase in cardiovascular diseases. Similarly, a reduction in LDL by 2mg/dl can result in 1% reduction in cardiovascular diseases (CVD). Phytochemical studies of the plants according to the literature reviewed, revealed the presence of saponin, and flavonoid (Dhanik *et al.*, 2017). Nutritional profile of the plants revealed the presence of vitamin C (Dhanik *et al.*, 2017); the slight reduction in LDL might be due to saponins in the extract. Since saponin is reported to

possess hypolipidemic potential. The mechanism by which saponin deplete LDL and TC have been suggested to be through the binding of bile acids with cholesterol forming strong insoluble complexes preventing its reabsorption (Iheanacho *et al.*, 2008). Vitamin C in the extract might also be the reason for the downward reduction although not significant since vitamin C is reported to cause reduction in LDL, TC and VLDL. The mechanism is due to the ability of vitamin C to activate 7 alpha-hydroxylase, the rate limiting enzymes that enhances the conversion of plasma cholesterol to bile acid thereby decreasing cholesterol levels.

CONCLUSION

Based on the results obtained in this study, it could be concluded that the combined extract of the two plants studied exerted hepatoprotective effect on the experimental animals and could be advantageous in treating hepatic disorders.

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