

Effect of Oral Administration of Aqueous Extract of Cloves (*Syzygium aromaticum*) on Serum Lipid Profile in Wistar Rats

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ABSTRACT

Effect of oral administration of aqueous extract of *Syzygium aromaticum* on serum lipid profile and atherogenic indices were assessed in 32 apparently healthy male Wistar rats. The animals were randomly grouped which were grouped and administered with different doses (mg/kg) as follows: Group A (normal diet without cholesterol-rich diet and extract), Group B (cholesterol-rich diet), Group C (cholesterol-rich diet and 100 mg/kg of aqueous extract of *S. aromaticum* (AESA)); Group D (cholesterol-rich diet and 200 mg/kg of AESA). Group E (cholesterol-rich diet and 400 mg/kg of AESA), Group F (normal diet and 100 mg/kg of AESA), Group G (normal diet and 200 mg/kg of AESA), Group H (normal diet and 400 mg/kg of AESA) for a period of four weeks. Animals that were fed with normal diet with the extract constituted the negative control group, and animal fed with cholesterol rich diet with the extract constituted the positive control group. At the end of the four weeks, the animals were sacrificed, serum lipid profile and Artherogenic indices were determined. Data was subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS 10.0) software. Duncan's Multiple Range Test was used to establish significant differences ($p < 0.05$). Results revealed that mean serum LDL-cholesterol, Total-cholesterol and Triglycerides were lower in animals fed normal diet and 400mg/kg of aqueous extract of *S. aromaticum* compared to animals fed with normal diet and 100 mg/kg. Thus, it could be inferred that "aqueous extract of *S. aromaticum*" especially at 400 mg/kg may lower some cardio risk factors due to its antihyperlipidemic effect. P values less than 0.05 were considered significant. Mean serum LDL, T. Chol and TRIG levels were lower in animals fed normal diet and 400 mg/kg of aqueous extract of *S. aromaticum* compared to animals fed with normal diet and 100 mg/kg. Thus, it could be suggested that "aqueous extract of *S. aromaticum*" especially at 400 mg/kg could reduce some of the risk factors of cardiovascular disease.

Keywords: *Syzygium aromaticum*, Lipid profile, Cholesterol, Atherogenic indices, Antilipidemic.

INTRODUCTION

Cholesterol is doubtless the most publicized lipid, notorious, because of the strong correlation between high levels of cholesterol in the blood and the incidence of the human cardiovascular disease (David and Michael, 2008). Epidemiological study (Henry *et al.*, 2003) has shown that, the level of total serum cholesterol is a strong predictor of the likelihood that an individual will develop cardiovascular disease. In evaluating the lipid-induced CHD risk, the level of LDL cholesterol is not as valuable as the LDL/HDL cholesterol ratio, which is the reliable criterion. Triglyceride levels make no independent contribution. Most CHD preventive measures, and improvement of glucose tolerance also increases HDL-cholesterol reduced levels of which are chiefly responsible for the LDL/HDL ratio in diabetics. Weight control merits a high priority because of its favourable influence on the lipid profile, glucose tolerance and blood pressure. Cardiovascular disease include a broad range of diseases, including hypertension, hyperlipidemia, thromboembolism, coronary heart disease, heart failure e.t.c. (Sadeghi, 2014). Hyperlipidemia is predisposing factor for many diseases that can cause complications such as atherosclerosis,

hypertension, increased risk of stroke, and fatty liver (Asgary, 2014).

The body can regulate lipoprotein levels by increasing or decreasing the production of lipoproteins. Lipid levels may become abnormal because of changes that occur with aging, various disorders (including inherited ones), as of certain drugs, lifestyle (such as consuming a diet high in saturated fat being physically inactive or overweight). Abnormal levels of lipids (especially cholesterol) can lead to long term problems, such as atherosclerosis. High levels of LDL-cholesterol with low levels of HDL-cholesterol increases the risk of atherosclerosis and thus the risk of heart attack and stroke (Golberg, 2017). Hypertension is the most common disorder and is known as a risk factor for the diagnosis of myocardial infarction, stroke, peripheral vascular disease and a major factor in the development of cardiovascular disease and mortality. Clove (*Syzygium aromaticum*) is one of the most valuable spices that have been used for centuries for many medicinal purposes. Considered a very strong spice due to the eugenol chemical that makes up most of the clove's taste (85 percent) (Falkowitz, 2011). Cloves are used in Indian Ayurvedic medicine, Chinese medicine and Western herbalism and

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dentistry where the essential oil is used as an anodyne (pain killer) for dental emergencies. Cloves are used as carminative to increase hydrochloric acid in the stomach and to improve peristalsis. Cloves are also said to be a natural antihelminthic (Balch and Balch, 2000).

The essential oil is used in aromatherapy when stimulation and warming are needed, especially for digestive problems. Cloves may be used internally as a tea and its essential oil topically for hypotonic muscles, including for multiple sclerosis (Balch and Balch, 2000) *Syzygium aromaticum* has many medicinal importance (Balch and Balch, 2000). However, most of these plants have been ingested indiscriminately by many local populations for the management of various diseases without knowing how they bring relief. It is imperative to note that there is dearth of information on the effect of consumption of aqueous extract of *Syzygium aromaticum* and incidence of cardiovascular diseases. Therefore, this study was aimed at evaluating the effect of *Syzygium aromaticum* consumption on serum lipid profile.

MATERIALS AND METHODS

Collection and Preparation of *Syzygium aromaticum* Extract

Cloves used for this research was obtained from Dawanau Market of Ungoggo Local Government Area of Kano State, Nigeria. The sample used was ground into powder, 50 grams of the pulverized sample was weighed in a conical flask and 500cm³ of distilled water was added. The top of the conical flask was shaken and covered with aluminium foil and was kept for 48 Hours at room temperature. The extract was obtained by filtration using Whatman No. 1 Filter paper and concentrated using vacuum evaporator.

Formulation of the cholesterol rich diet

The cholesterol rich diet was formulated according to the method of Vesselinvitch *et al.* (1980) which is as follows: Pure cholesterol powder 2% and 20% palm oil were thoroughly mixed with grower mash feeds.

Animals and Housing

Male wistar rats weighing 120-150g were obtained from the Animal House of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University (ABU) Zaria. The wistar rats were maintained on starter feeds (produced by vital feeds Nigeria), and water. The animals were housed in animal house of Zoology Department Bayero University, Kano in propylene cages at room temperature throughout the study

Treatment of Experimental Animals (Rats)

Thirty-two (32) adult experimental male wistar rats were divided into eight groups of four (4) animals each and treated as follows:

- I. Group A rats were fed with normal diet without cholesterol-rich diet and extract.
- II. Group B rats were fed with cholesterol-rich diet.
- III. Group C rats were fed with cholesterol-rich diet and 100mg/kg of aqueous extract of *Syzygium aromaticum* (AESA).

- IV. Group D rat were fed with cholesterol-rich diet and 200mg/kg of AESA.
- V. Group E rats were fed cholesterol-rich diet and 400mg/kg of AESA.
- VI. Group F rats were fed with normal diet and 100mg/kg of AESA.
- VII. Group G rats were fed with normal diet and 200mg/kg of AESA.
- VIII Group H rats were fed with normal diet and 400mg/kg of AESA.

Determination of Lipid Profile

The serum triglycerides (TRIG) were determined using (Jacobs and Von Denmark, 1960), very low density lipoprotein (VLDL) and total cholesterol (T.Chol) levels were measured using (Allain *et al.*, 1974). Determination of serum high density lipoprotein (HDL-Chol) fraction were determined using (Lopes-virella *et al.*, 1977), and serum low density lipoprotein (LDL) level was estimated using (Friedwald, 1972).

Estimation of Serum LDL-Cholesterol

Serum LDL-Cholesterol estimated according to Friedwald *et al.* (1972) using the equation below:

$$\text{LDL - Cholesterol} = \text{Total Cholesterol} - \left[\frac{\text{TAG}}{5} + \text{HDL - Cholesterol} \right]$$

Estimation of VLDL- Cholesterol

The concentration of VLDL- Cholesterol was determined as follows:

$$\text{VLDL - Cholesterol(mg/dl)} = \frac{\text{Triglyceride}}{5}$$

Determination of Artherogenic indices

Artherogenic indices were calculated as follows:

$$\text{Cardio Risk ratio} = \frac{\text{Total cholesterol}}{\text{HDL-Cholesterol}}$$

$$\text{Artherogenic coefficient} = \frac{\text{Total-cholesterol-HDL-Cholesterol}}{\text{HDL-Cholesterol}}$$

Data collected were subjected to one-way analyses of variance (ANOVA) using the General Linear Model (SPSS for Windows).

RESULTS AND DISCUSSION

Serum total cholesterol (T.Chol), HDL-Cholesterol (HDL-Chol), Triglycerides (Trig), low density lipoprotein cholesterol (LDL-cholesterol), and very low density lipoprotein cholesterol (VLDL-cholesterol) levels of Wistar rats administered with 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight of aqueous extract of *Syzygium aromaticum* (AESA) for twenty days (28) days are presented in Table 1. The mean serum HDL-cholesterol level of negative control rats (Group I) was significantly higher ($p < 0.05$) compared to animals fed with normal diet and 100 mg/kg (Group VI). The mean serum HDL-cholesterol of animals fed with normal diet and 100 mg/kg of AESA (Group VI) was significantly lower ($p < 0.05$) compared and 400 mg/kg of AESA (Group VIII).

Furthermore, the mean serum LDL cholesterol level of all the groups showed no significant difference except animals fed with normal diet and 100 mg/kg of AESA (Group VI) showed significant ($p < 0.05$) increase compared to animals fed with normal diet and 400 mg/kg of AESA (Group VIII). The mean serum VLDL-cholesterol of animals fed with normal diet and 100 mg/kg of AESA (Group VI), increases compared to animals fed with normal diet and 400 mg/kg of AESA (Group VIII). The study also showed that the mean serum triglyceride levels of animals fed with normal diet and 100 mg/kg of AESA (Group VI), increases compared to animals fed with normal diet and 400 mg/kg of AESA (Group VIII). Atherogenic indices like cardiac risk ratio and atherogenic coefficient were also determined. The study shows that both the cardiac risk ratio and atherogenic coefficient for group V (animals fed with cholesterol rich diet and 400 mg/kg of AESA) and group VIII (animals fed with normal diet and 400 mg/kg of AESA) decreases as compared to groups III, IV, VI and VII.

The result shows that AESA has antihyperlipidemic effect and is dose dependent. Several studies have indicated that diet treatment or drug therapy to regulate cholesterol can reduce subsequent cardiovascular diseases associated morbidity and mortality (Kwiterovich, 1997). The crucial risk factor of cardiovascular diseases (CVD) includes low level of HDL – cholesterol. HDL-cholesterol inhibits 1, 2-lipoxygenase-mediated formation of lipid hydro peroxides (Nofer, 2002). Low level of HDL – Cholesterol plays a

direct role in the atherogenic process, therapeutic intervention to raise HDL – Cholesterol together with other risk factors are widely encouraged (LRCP, 1984). Hypercholesterolemia has long been recognised as a major factor for coronary artery diseases and myocardial infarction (Eisenberg, 1998).

Several studies have demonstrated that hypercholesterolemia markedly increases myocardial infarctions reperfusion injury as evidenced by increased apoptotic and necrotic cell death, and infarct size (Sakamoto, 1991). Many experimental and clinical studies have demonstrated that hypercholesterolemia is associated with increased inflammatory responses, such as enhanced endothelial adhesion molecule expression and oxidative stress. It could be suggested that AESA could probably reduce some of the risk factors of cardiovascular disease at a dose of 400 mg/kg. The increased HDL-cholesterol level and decrease LDL-cholesterol level may decrease the risk of cardiovascular disease.

In this study *Syzygium aromaticum* led to significant elevation of HDL-cholesterol and decreases LDL, VLDL, total cholesterol, and triglyceride at dose of 400 mg/kg, indicating a promising role against cardiovascular disease.

Table 1 presents the results of serum lipid profile, after oral administration of 100, 200 and 400 mg/kg body weight respectively of AESA for twenty eight (28) days.

Table 1: Lipid profile of rats after oral administration of various dosages of aqueous extract of *Syzygium aromaticum* (AESA) for 28 days.

Group	Dose (mg/kg)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	Cholesterol (mmol/L)	Trig. (mmol/L)
I	0	2.63±0.06 ^a	0.99±1.05	0.27±0.03 ^g	3.93±0.83 ⁱ	0.60±0.08 ^k
II	0	2.84±0.51	1.36±1.10	0.36±0.24	4.63±1.40	0.80±0.52
III	100	1.67±1.09	2.39±1.23	0.27±0.03	4.37±1.21	0.60±0.06
IV	200	1.92±0.15	3.46±2.11	0.21±0.01	5.63±2.04	0.46±0.03
V	400	2.50±0.94	1.90±1.66	0.34±0.09	4.79±1.27	0.75±0.21
VI	100	1.59±0.23 ^{bc}	4.74±3.01 ^e	0.81±0.7 ^g	8.17±3.12 ⁱ	0.76±0.13 ^k
VII	200	2.008±0.43	1.51±1.06	0.43±0.21 ^g	4.08±1.10 ⁱ	0.95±0.46 ^k
VIII	400	2.52±0.40 ^d	0.25±0.26 ^f	0.35±0.14 ^h	2.24±0.37 ^j	0.79±0.31 ^l

Values are presented as mean ± standard deviation (mean ± SD). Values in the same column with different superscript are significantly different at $p < 0.05$ compared with the control. $n = 32$, that is, 4 Wistar rats for each group. T. Chol = Total Cholesterol; HDL = High Density lipoproteins; Trig. = Triglycerides; VLDL = Very Low Density Lipoproteins; LDL = Low Density Lipoproteins;

Table 2 depicts the results on atherogenic indices after oral administration of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of AESA for twenty (28) days. The atherogenic coefficient and cardiac risk ratio of animals fed with cholesterol rich diet and 400 mg/kg of AESA (Group V), and animals fed with normal diet and 400 mg/kg of

AESA (Group VIII), were significantly lower compared to animals fed with cholesterol rich diet and 100 mg/kg of AESA (Group III), animals fed with cholesterol rich diet and 200 mg/kg AESA (Group IV), animals fed with normal diet and 100 mg/kg AESA (Group VI), animals fed with normal diet and 200 mg/kg AESA (Group VII).

Table 2: Atherogenic indices of Wistar rats after oral administration of various dosages of aqueous extract of *Syzygium aromaticum* (AESA) for 28 days

Group	Dose(mg/kg)	Atherogenic coefficient	Cardiac risk ratio
I	0	0.49	1.49
II	0	0.63	1.63
III	100	1.62	2.62
IV	200	1.93	2.93
V	400	0.92	1.92
VI	100	4.14	5.14
VII	200	1.03	2.03
VIII	400	-0.11	0.89

Values -0.3 to 0.01 for low risk cardiovascular disease (CVD); 0.1 to 0.24 for medium risk CVD; >0.24 for high risk CVD (Dobiasova, 2006).

Figure 1 depicts the results of atherogenic coefficient and cardiac risk ratio of Wistar rats after oral administration of 100, 200 and 400 mg/kg body weight respectively of AESA twenty eight (28) days.

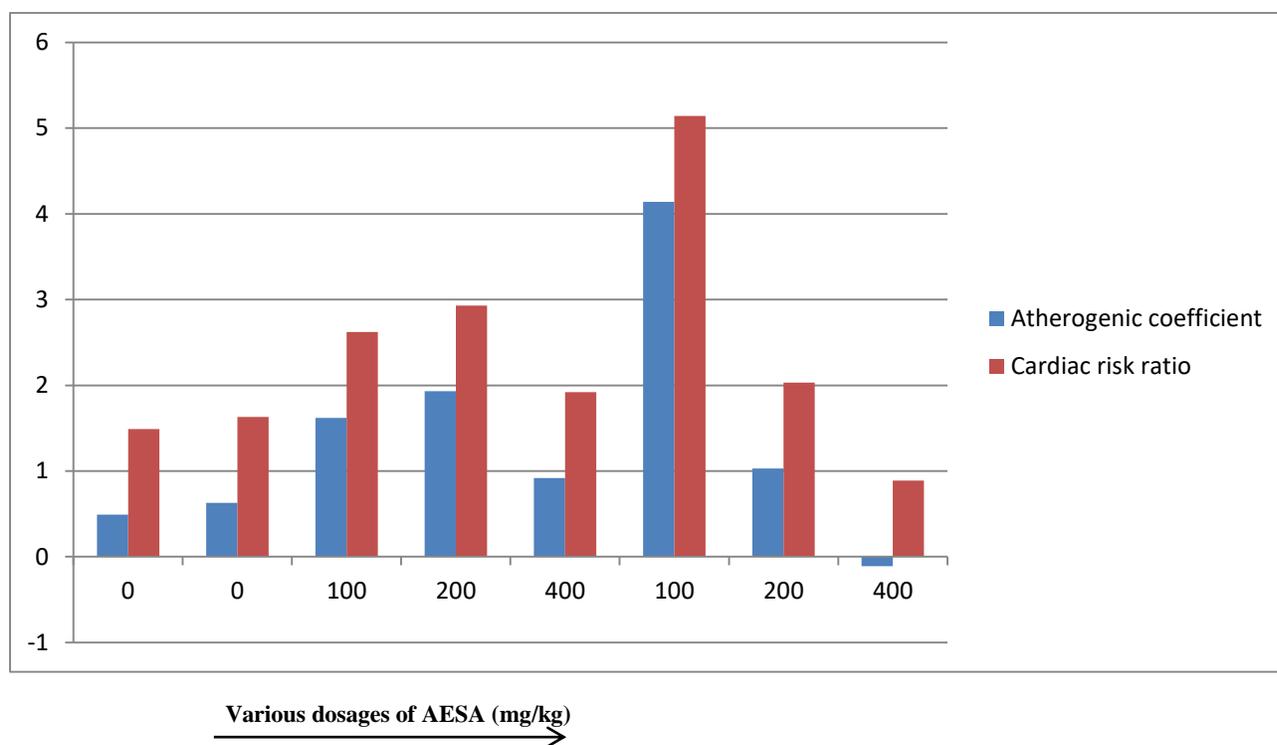


Figure 1: Atherogenic coefficient and cardiac risk ratio of Wistar rats given various dosages (100 – 400 mg/kg) of AESA

The result of this study shows that AESA has antihyperlipidemic effect on diet induced hypercholesterolaemia in Wistar rats and is dose dependent.

Author's contribution

The first author S.A Bichi carried out the research under the supervision of M.S. Sule, M. Dalhatu assisted in the laboratory work.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCES

- Asgary, S., Rafiean-Kopei, M., Shamsi, F., Najali, S. and Sahebkar, A. (2014). Biochemical and Histopathological study of the hyperglycemic and anti-hyperlipidemic effects of Cornelian cherry in alloxan induced diabetic in rats. *Journal of Complementary and Integrative Medicine*. **11**(2):63-69.
- Balch, P. and Balch, J. (2000). *Prescription for Nutritional Healing*, (3rd ed). Avery Publishers New York. P 94.
- Dobiasova, M (2006). AIP-atherogenic index of plasma as a significant predictor of cardiovascular risk: from research to practice. *Vnitri lekarstvi*. **52**(1): 64-71.

- David, L.N. and Michael, M.C. (2008). *Principle of Biochemistry*. Fifth edition. W.H. Freeman and company, New York. Pp.831-834.
- Eisenberg, D.A. (1998). Cholesterol lowering in the management of coronary artery disease: the clinical implications of recent trails. *American Journal of Medicine*: 104:25-55
- Friedewald, W. T., Levy, R.I and Fredrickson, D.S. (1972). Estimation of the concentration of LDL-cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*. **18**(6):499-515.
- Goldberg, M.D. (2017). A new era in the prevention of cardiovascular heart disease. *American Heart Journal. Symposium report on lipid regulation*. **110** (5):100-1107.
- Henry, R., Black, F., Mike, S. and David, N. (2003). Cardiovascular risk factors. *American Journal of Nutrition*, 76: 93-99.
- Kwiterovich, P.O. (1997).The effect of dietary fat,antioxidants,and pro-oxidants on blood lipids, lipoproteins,and atherosclerosis. *Journal of the American Dietetic Association.*, 97:31-41.
- Lopes-Virella, M. F., Stone, P. and Ellis, S. (1977). Cholesterol Determination in High-density Lipoprotein separated by three different methods. *Clinical Chemistry*. 23: 882.
- LRCP (1984): Lipid Research Clinics Program. The lipid research clinics,coronary primary prevention trial results Reduction in incidence of coronary heart disease. *Journal of the American. Medicine Association*. 251: 351-364
- Nofer, J.R.,Kehrel, B. and Fobker, M. (2002). HDL and atherosclerosis beyond reverse cholesterol transport.*Artherosclerosis*. 161: 1-16.
- Allain, C.C, Poon, I.S., Chan, C.H.G., Richmond, W. and Fu, P.C. (1974).Enzymatic determination of serum total cholesterol. *Clinical Chemistry*. 20:470-471)
- Sadeghi, M., Khosravi-Boroujeni, H., Sarrafzadegan, N., Asgary, S., Roohofza,H. and Gharipour, M.(2014). Cheese consumption in relation to cardiovascular risk factors among Iranian adults IHHP Study. *Nutrition Research and practice* **8**(3):336-341.
- Sakamoto, S., Kashiki, M., Imai, N., Liang, C.S. and Hood, W.B. Jr (1991). Effect of short-term, diet-induced hypercholesterolemia on systemic hemodynamics myocardial blood flow, infarct size in awake dogs with acute myocardial infarction. *Circulation*. 84(1): 378-386)
- Vesselinitch, D., Wissler, R.W., Schiffner, T.T. and Borenaztjin, I. (1980). The effect of various diet on atherogenesis in rhesus Monkey. *Artherosclerosis*. 35: 189-207.