Majalah Obat Tradisional (Trad. Med. J.), January-April 2025
Vol. 30(1), p 61-66
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 21-03-2024
Revised : 14-07-2024
Accepted : 03-10-2024

Anti-hyperlipidemic and Biochemical Effect of Aqueous Extract of *Combretum dolichopetalum* in High Cholesterol Diet Fed Rats

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ABSTRACT

It is commonly known that atherosclerosis and coronary heart disease (CAD) are associated with hyperlipidemia, which is brought on by abnormalities in the transportation, production, or catabolism of cholesterol. Hence, preventing hyperlipidemia can positively impact managing and treating cardiovascular diseases. This present study aimed to examine the possible aqueous extract's effects of *Combretum dolichopetalum* (AECD) on liver indices and lipid status in rats on a diet rich in cholesterol. Five groups of twenty-five male albino Wistar rats, weighing between 130 and 160 grams, were assembled. Group A (Standard control group), Group B (Hyperlipidemic control), Group C (treatment): which received high cholesterol diet (HCD) + AECD (200 mg/kg bwt.), Group D: HCD + AECD (400 mg/kg bwt), Group E: HCD + atorvastatin (standard drug) (30 mg/kg bwt). The rats were sacrificed on day 28th of the experiment, and samples were obtained for liver indices and lipid profile using standard protocols. The findings show that the treatment groups' lipid profile was significantly lower ($p < 0.05$) than those of the hyperlipidemic controls. The liver enzymes; alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and total bilirubin (TB), direct bilirubin (DB), remain unchanged across all groups. These findings suggest that AECD is beneficial in lowering cholesterol levels and does not have a toxic effect on the liver.

Keywords: *Combretum dolichopetalum*; hyperlipidemia; liver enzymes; lipid profile

INTRODUCTION

Hyperlipidemia is abnormally high blood levels of lipids, a main cause of cardiovascular disease risk (Amit *et al.,* 2011; Dhaliya *et al.,* 2013; Mainieri et al., 2023). According to Dhaliya *et al.* (2013), there is a specific elevation of lipid profile in plasma in this situation. Studies have shown that consuming a lot of saturated fat acids (SFAs) in your diet and eggs (which contain high levels of cholesterol and saturated fat) raises blood cholesterol levels more than other dietary factors (Baumgartner *et al.,* 2013; Jacobson *et al.,* 2015). Additionally, this may raise the chance of developing diabetes and other cardiometabolic illnesses, such as cardiovascular disease (CVD) (Weggemans *et al.,* 2001; Spence *et al.,* 2012; Li *et al.,* 2013). Lowering the risk of ischemic heart disease, the development of other cardiovascular or cerebrovascular illnesses is the main goal of managing or treating hyperlipidemia (Davey *et al.,* 1992).

However, using synthetic medications can have a number of negative consequences, including flushing, dry skin, hyperuricemia, diarrhea, nausea, myositis, and altered liver function (Davey *et al.,* 1992). Plants have long been utilized as a rich source of secure and efficient medications, and herbal remedies have long been the major source of primary healthcare worldwide (Amaechi, 2009). The effectiveness, safety, affordability, and acceptability of herbal medicines have been well-reported (Muramatsu *et al.,* 1986; Balkrishna *et al.,* 2024). Some herbs are known to reduce high blood cholesterol levels with no side effects and at a lower cost.

Combretum dolichopetalum (CD) is a climber that is dicotyledonous and typically found in savannah areas (Hutchinson and Dalziel, 1954). Scientific literature has extensively documented

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the plant's medical applications: Antitrypanosomal and antiplasmodial activity (Atindehou *et al.,* 2004; Muthu *et al.,* 2006; Nnadi *et al.,* 2022), antiinflammatory activity (Gansane *et al.,* 2010), treatment of anaemia (Emelike *et al.,* 2021) and management of diabetics (Emelike *et al.,* 2023). The CD leaf contains flavonoids, alkaloids, steroids, and macronutrients (Uzor *et al.,* 2015; Emelike *et al.,* 2020). We have previously utilized gas chromatography-mass spectrometry (GC-MS) to examine the plant's chemical constituents (Emelike *et al.,* 2021). The plant is traditionally used for medicinal purposes to alleviate or cure stomach pains, diarrhoea, diabetics, gastrointestinal diseases, and blood in the stool (Asuzu and Njoku, 1992; de Morais Lima *et al.,* 2011; Emelike *et al.,* 2022; Emelike *et al.,* 2023). In this investigation, we assessed the anti-hyperlipidemic impact of aqueous extract *Combretum dolichopetalum* (AECD) on the liver and lipid profile of rats fed a diet high in fat.

MATERIALS AND METHODS

Materials

Plant materials

In 2017, *C. dolichopetalum* leaves that had just reached maturity were collected from the plant's native environment in Nsukka, Enugu State, Nigeria. The taxonomist that recognized the plant samples was C. J. Onyeukwu, from the University of Nigeria, Department of Plant Science and Biotechnology. The sample voucher of the plant, UNH No. 49a, was kept for later use in the herbarium.

Preparation of extract

Following a thorough cleaning and seven days of air drying at room temperature, an electric blender (model ms-233, China) was used to grind the leaves into a coarse powder. Using a 30 to 70 aqueous solution ratio of water and ethanol as the solvent, two (2.0) kilograms of the powdered material were added to the extraction chamber of the soxhlet extractor in accordance with Jensen's (2007) method. The extraction temperature was kept constant at 100°C for a total of 48 hours. Once the 48 hours were up, the extract that was collected in water was dried in a lab oven at a low temperature of 40°C. This resulted in a pasty, dark-green extract with a distinct aromatic fragrance. The extract was then weighed and stored safely in a refrigerator for future use.

Methods

Animal experiments

A total of twenty-five (25) healthy male Wistar albino rats weighed between 130 and 160g were taken from the Department of Physiology's animal house at Alex Ekwueme Federal University Ndufu-Alike (AE-FUNAI), Ebonyi State. Two weeks prior to the start of the trial, the rats were given the opportunity to get used to their water and their feed (Vital feed®, Nigeria).

Rodents were kept in conventional conditions, having a 12-hour day/night cycle and 25 ± 2°C as its temperature. The Department of Physiology's Research Ethics Committee at AE-FUNAI accepted the protocol for this study, which has protocol number PHS/EC/02/02/2017. The NIH standards for the use of experimental animals and established institutional protocols were followed in the conduct of the animal study.

Induction of hyperlipidemia

In order to induce hypercholesterolemia, rats were fed a diet containing 1% cholesterol, cholic acid (0.2%), methyl thiouracil (0.2%), sodium chloride (1%), lard (4%), egg yolk (7%). Others include wheat bran (6.6%), corn starch (35%), and wheat flour (45%) for a period of six weeks. This protocol was outlined by Pengzhan *et al.* (2003).

Animal grouping

The study involved five groups of rats, each with a different treatment: Group A: Standard control rats received distilled water; Group B: Hyperlipidemic control rats received distilled water; Group C: Hyperlipidemic rats received 200 mg/kg bwt of AECD; Group D: Hyperlipidemic rats received 400 mg/kg bwt of AECD; Group E: Hyperlipidemic rats treated with atorvastatin (a standard drug) (30 mg/kg bwt).

The doses of the extracts used in the study were chosen based on acute toxicity testing conducted by Emelike *et al.* (2020). Even at a high dosage of 5000 mg/kg body weight, the tests revealed that the extract was not harmful. Using an oropharyngeal cannula, the extract was given orally to the rats once daily for 28 days, during which their body weights were monitored continuously. The rats were starved overnight and given access to solely drinking water following the extract delivery time. A 2% sodium pentobarbital intraperitoneally (75 mg/kg) was then administered to put them under anesthesia. For the investigation of the lipid profile and liver function activities, approximately 4 milliliters of venous blood were drawn using the orbital method and placed into plain tubes.

Lipid profile

Using Biosystems kits and fully automated A25 Biosystem equipment, the lipid profile

Table I. Effect of AECD on rats' body weights

Groups	Initial Weight (g)	Final Weight (g)	Weight Difference(g)	% Change in Body Weight
Group A	183.1 ± 2.59	218.1 ±3.17	35.2 ± 3.25	16.9 ± 3.61
Group B	183.2 ± 3.02	231.0 ± 2.06	48.4 ± 3.21[a]	20.9 ± 3.02[a]
Group C	188.9 ± 3.16	201.0 ± 3.04	12.1 ± 3.42[ab]	5.8 ± 3.13[ab]
Group D	186.7 ± 2.91	199.0 ± 2.01	11.7 ± 2.55[ab]	5.4 ± 2.78[ab]
Group E	189.9 ± 3.16	200.0 ± 16.4	10.1 ± 3.40[ab]	4.8 ± 3.13[ab]

Values represent mean ± SEM. Significance indicated as a = $p<0.05$ vs standard control (Group A) and b = $p<0.05$ vs hyperlipidaemic control (Group B).

Table II. Effect of AECD on lipid profile

Groups	TC (mmol/l)	TG (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)
Group A	1.77 ± 0.04	0.31 ± 0.70	1.14 ± 0.14	0.63 ± 0.10
Group B	3.02 ± 0.09[a]	1.71 ± 0.68	1.03 ± 0.09	1.98 ± 0.05[a]
Group C	2.67 ± 0.10[a]	0.97 ± 0.07	1.13 ± 0.06	1.56 ± 0.04[a]
Group D	2.35 ± 0.10[ab]	0.74 ± 0.07	1.14 ± 0.08	1.19 ± 0.06[b]
Group E	2.10 ± 0.25[b]	0.42 ± 0.03	1.19 ± 0.23	0.91± 0.32[b]

Values represent mean ± SEM. Significance indicated as a = $p<0.05$ vs standard control (Group A) and b = $p<0.05$ vs hyperlipidaemic control (Group B).

Table III. Effect of AECD on liver profile

Groups	TB (mg/dL)	CB (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
Group A	10.90 ± 1.60	5.89 ± 1.00	21.0 ± 0.59	77.0 ± 7.93	99.6 ± 4.97
Group B	7.01 ± 1.61	3.68 ± 0.10	15.2 ± 2.12	53.1 ± 2.46	96.0 ± 2.63
Group C	6.30 ± 0.76	3.83 ± 0.21	18.1 ± 2.46	47.6 ± 3.50	100.2 ± 7.20
Group D	6.87 ± 0.66	4.26 ± 0.40	17.3 ± 2.70	59.0 ± 9.40	102.1 ± 9.99
Group E	8.92 ± 1.54	5.18 ± 0.66	17.7 ± 2.13	50.6 ± 8.83	111.0 ± 4.79

Values represent mean ± SEM. Significance indicated as a = $p<0.05$ vs standard control (Group A) and b = $p<0.05$ vs hyperlipidaemic control (Group B).

[triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C)] in rats were determined. The low-density lipoprotein cholesterol (LDL-C) was estimated using the formula below:

$$\text{LDL (mg/dl)} TC - \frac{TG}{5} - \text{HDL (Friedwald, 1972)}$$

Liver function assays

Rats' serum activity for ALT, AST, ALP, TB and DB were assessed using fully automated A25 Biosystem devices.

Statistical analysis

The GraphPad Prism software was used to analyze the data that was collected. The data were compared using one-way ANOVA subsequent to the Turkey post hoc test. Our definition of significant was a value of $p<0.05$. Mean ± standard error of the mean (SEM) was used to present the results.

RESULTS

Table I shows the effect of repeated administration of AECD on body weight (beginning weight, ending weight, difference in weight, and percentage change in body weight). The hyperlipidemic group experienced a statistically significant rise in body weight in comparison to the control group. However, there was a decrease in the body weight of animals treated with AECD. Table II demonstrates that the hyperlipidemic group (3.02 ± 0.09) had a significantly higher total cholesterol level ($p<0.05$) than the standard group (1.77 ± 0.04). Comparing the total cholesterol levels of groups C (2.67 ± 0.10) and D (2.35 ± 0.10) to the hyperlipidemic group (3.02 ± 0.09), there was a substantial ($p<0.05$) drop in total cholesterol. From Table II, there appeared to be a decrease in the concentration of TG in the treated groups C (0.97 ± 0.68) and D (0.74 ± 0.07) when compared with the hyperlipidemic group (1.71 ± 0.68), although not significant. In this study, there was no significant change in the liver indices in all the groups in Table III.

DISCUSSION

This decrease in the body weight of animals treated with AECD may be due to the plant's saponin content, which has been previously

investigated (Emelike *et al.,* 2021). Research has shown that saponins reduce appetite (Kim *et al.,* 2005).

This result of hyperlipidemic activity is consistent with a study by Dhulasavant *et al.* (2010), which found that giving high-cholesterol meals enhanced with eggs and coconut oil to animals led to a considerable increase in the animals' serum cholesterol levels. This result demonstrates the leaf extract's ability to reduce the rats' total cholesterol levels. The anti-hyperlipidemic effects of AECD could be because of the existence of some phytochemical constituents in the leave extract, such as flavonoids, tannins and saponins activity (Olagunju *et al.,* 1995; Emelike *et al.,* 2021).

According to Nagao *et al.* (2018), there is a cardioprotective benefit associated with higher HDL-C levels. Treatment with AECD, as shown in Table II, did not potentiate any significant change in HDL-C. However, the levels of LDL-C showed a significant decrease in the test groups C and D when compared with the hyperlipidemic group. Research has reported that flavonoids have increased HDL-C concentration and decreased LDL-C and VLDL levels in hypercholesteraemic rats (Sun *et al.,* 2021). Flavonoids found in AECD (Emelike *et al.,* 2020) could be considered responsible for increasing HDL-C and decreasing LDL-C in this study.

This study of the liver indices is consistent with the findings of Emelike *et al.,* 2020, that administering AECD had no effect on blood levels of AST, ALT, and ALP or AST and ALP. This finding implies that neither our high-fat diet nor AECD has any toxic effect on the liver biomarkers.

CONCLUSION

In summary, research has demonstrated that AECD is safe for the liver and effective in lowering cholesterol levels.

ACKNOWLEDGEMENTS

The Department of Physiology at the Alex Ekueme Federal University Ndufu-Alike, Ebonyi State, Nigeria, has our sincere gratitude.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Amaechi, N.C. (2009). Nutritive and Anti-nutritive evaluation of wonderful kola *(Buccholzia coricea*) seeds. *Parkinson Journal of Nutrition,* 8(8):1120-1122. https://scialert.net/abstract/?doi=pjn.2009.1120.1122

Amit, G., Vandana, S. & Sidharth, M. (2011). Hyperlipidemia. *International Journal of Biopharmacology and Toxicology Research,* 1:81-89.

Asuzu, I. U. & Njoku, J. C. (1992). The pharmacological properties of the ethanolic root extract of *Combretum dolichopetalum*. *Phytotherapy Research*, 6:125-8.

Atindehou, K. K., Schmid, C., Brun, R., Kone, M. W. & Traore, D. (2004). Antitrypanosomal and antiplasmodial activity of medicinal plants from Cote d'Ivoire. *Journal of Ethnopharmacology*, 90:221–227.

Balkrishna, A., Sharma, N., Srivastava, D., Kukreti, A., Srivastava, S. & Arya, V. (2024). Exploring the safety, efficacy, and bioactivity of herbal medicines: bridging traditional wisdom and modern science in healthcare. *Future Integrative Medicine*, 3(1):35-49.

Baumgartner, S., Kelly, E.R., vander Made, S., Berendschot, T.T., Constance, H. & Lutjohann, D. (2013). The influence of consuming an egg or an egg-yolk buttermilk drink for 12 wk on serum lipids, inflammation, and liver function markers in human volunteers. *Nutrition*, 29:1237-1244. https://doi.org/10.1016/j.nut.2013.03.020

Davey Smith, G., & Pekkanen, J. (1992). Should there be a moratorium on the use of cholesterol lowering drugs?. *BMJ (Clinical research ed.)*, *304*(6824), 431–434. https://doi.org/10.1136/bmj.304.6824.431

de Morais Lima, G. R., de Albuquerque Montenegro, C., de Almeida, C. L., de Athayde-Filho, P. F., Barbosa-Filho, J. M., & Batista, L. M. (2011). Database survey of anti-inflammatory plants in South America: a review. *International Journal of Molecular Sciences*, *12*(4), 2692–2749. https://doi.org/10.3390/ijms12042692

Dhaliya, S. A., Surya, A. S., Dawn, V. T., Betty, C., Arun, K. & Sunil, C. (2013). A review of hyperlipidemia and medicinal plants. *International Journal of Applied Pharmaceutical Sciences and Biological Sciences,* 2(4): 219-237.

Dhulasavant, V., Shinde, S., Pawar, M., & Naikwade, N. S. (2010). Antihyperlipidemic activity of *Cinnamomum tamala* Nees, on high cholesterol diet induced hyperlipidemia. *Int J Pharm Tech Res.,* 2:2517–21.

Emelike, C. U., Anyaehie, U. S. B., Iyare, E. E., Obike, C. A., Aloke, C., Chukwu, D. F., Eleazu, C. O., Chukwu, C. J., Ekakitie, O. O., Konyefom, N. G.,

Uzomba, C. G., Chukwu, J. A. O. (2021). Chemical composition and evaluation of methanol leaf extract of *Combretum dolichopetalum* on body weights and haematological indices of phenylhydrazine induced-anaemic rats. *Toxicology International,* 28(2):135-44. https://www.informaticsjournals.com/index.php/toxi/article/view/26603

Emelike, C. U., Anyaehie, U. S. B., Iyare, E. E., Obike, C. A., Aloke, A., Ekakitie, O. O., Chukwu J. A. O., Maduka N. & Chukwu, O. O. (2022). Effect of prenatal consumption of *Combretum dolichopetalum* by pregnant rats on haematological, biochemical parameters and pregnancy outcome. *Physiology and Pharmacology (Iran)*, 26(4) 459–467 http://ppj.phypha.ir/article-1-1756-en.html

Emelike, C. U., Chijioke-Agu, O. E., Nwoke, F.-A. K., Oyibe, R., Enwereuzo, C. G. & Ahuekwe, E. F. (2023). Modulation of antioxidant activities, markers of hepatic and renal dysfunctions in alloxan-induced diabetic rats by *Combretum dolichopetalum. Notulae Scientia Biologicae*, *15*(3), 11315. https://doi.org/10.55779/nsb15311315

Emelike, C.U., Anyaehie, U. S. B., Iyare, E. E., Obike, C. A., Eleazu, C. O. & Chukwuma, C. (2020). Acute and sub-acute toxicity studies on *Combretum dolichopetalum* Engl. & Diels leaves. *Slovenian Veterinary Research,* 57 (3): 105–114. https://doi.org/10.26873/SVR-899-2020

Gansané, A., Sanon, S., Ouattara, L. P., Traoré, A., Hutter, S., Ollivier, E., Azas, N., Traore, A. S., Guissou, I. P., Sirima, S. B., & Nebié, I. (2010). Antiplasmodial activity and toxicity of crude extracts from alternatives parts of plants widely used for the treatment of malaria in Burkina Faso: contribution for their preservation. *Parasitology research*, *106*(2), 335–340. https://doi.org/10.1007/s00436-009-1663-y

Hutchinson, J. & Dalziel, J.M. (1954). Flora of West Tropical Africa. Vol. I, Part 1, 2nd Edition, Crown Agents for Overseas Governments Administrations, London.

Jacobson, T. A., Maki, K. C., Orringer, C. E., Jones, P. H., Kris-Etherton, P., Sikand, G., La Forge, R., Daniels, S. R., Wilson, D. P., Morris, P. B., Wild, R. A., Grundy, S. M., Daviglus, M., Ferdinand, K. C., Vijayaraghavan, K., Deedwania, P. C., Aberg, J. A., Liao, K. P., McKenney, J. M., Ross, J. L., … NLA Expert Panel (2015). National lipid association recommendations for patient-centered management of dyslipidemia: Part 2. *Journal of clinical lipidology*, *9*(6 Suppl), S1–122.e1. https://doi.org/10.1016/j.jacl.2015.09.002

Kim, J. H., Hahm, D. H., Yang, D. C., Kim, J. H., Lee, H. J., & Shim, I. (2005). Effect of crude saponin of Korean red ginseng on high-fat diet-induced obesity in the rat. *Journal of pharmacological sciences*, *97*(1), 124–131. https://doi.org/10.1254/jphs.fp0040184

Li, Y., Zhou, C., Zhou, X., & Li, L. (2013). Egg consumption and risk of cardiovascular diseases and diabetes: a meta-analysis. *Atherosclerosis*, *229*(2), 524–530. https://doi.org/10.1016/j.atherosclerosis.2013.04.003

Mainieri, F., La Bella, S. & Chiarelli, F. (2023). Hyperlipidemia and cardiovascular risk in children and adolescents. *Biomedicines*, 11(3):809. https://doi.org/10.3390/biomedicines11030809

Muramatsu, K., Fukuyo, M., & Hara, Y. (1986). Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *Journal of nutritional science and vitaminology*, *32*(6), 613–622. https://doi.org/10.3177/jnsv.32.613

Muthu, C., Ayyanar, M., Raja, N., & Ignacimuthu, S. (2006). Medicinal plants used by traditional healers in Kancheepuram district of Tamil Nadu, India. *Journal of ethnobiology and ethnomedicine*, *2*, 43. https://doi.org/10.1186/1746-4269-2-43

Nagao, M., Nakajima, H., Toh, R., Hirata, K. I., & Ishida, T. (2018). Cardioprotective effects of high-density lipoprotein beyond its anti-atherogenic action. *Journal of atherosclerosis and thrombosis*, *25*(10), 985–993. https://doi.org/10.5551/jat.RV17025

Nnadi, C. O., Onuku, R., Ayoka, T. O., Okorie, N. H. & Nwodo N. J. (2022). Chemical constituents of *Combretum dolichopetalum:* Characterization, antitrypanosomal activities and molecular docking studies*. Tropical Journal of Pharmaceutical Research,* 21 (4): 801-808 http://www.tjpr.org http://dx.doi.org/10.4314/tjpr.v21i4.17

Olagunju, J.A., Ogunlana, C.O. & Gbile, Z.O. (1995). The hypoglycemic activity of ethanolic extracts of unripe, mature fruits of *Carica papaya* in alloxan-induced diabetic rats. Nigerian *Journal of Biochemistry and Molecular Biology*, 10:21-28.

Pengzhan, Y., Ning, L., Xiguang, L., Gefei, Z., Quanbin, Z., & Pengcheng, L. (2003). Antihyperlipidemic effects of different

molecular weight sulfated polysaccharides from *Ulva pertusa* (Chlorophyta). *Pharmacological research*, *48*(6), 543–549. https://doi.org/10.1016/s1043-6618(03)00215-9

Spence, J. D., Jenkins, D. J., & Davignon, J. (2012). Egg yolk consumption and carotid plaque. *Atherosclerosis*, *224*(2), 469–473. https://doi.org/10.1016/j.atherosclerosis.2012.07.032

Sun, J., Wang, Z., Chen, L., & Sun, G. (2021). Hypolipidemic effects and preliminary mechanism of chrysanthemum flavonoids, its main components luteolin and luteoloside in hyperlipidemia rats. *Antioxidants (Basel, Switzerland)*, *10*(8), 1309. https://doi.org/10.3390/antiox10081309

Uzor, P. F., Ebrahim, W., Osadebe, P. O., Nwodo, J. N., Okoye, F. B., Müller, W. E., Lin, W., Liu, Z., & Proksch, P. (2015). Metabolites from *Combretum dolichopetalum* and its associated endophytic fungus *Nigrospora oryzae*-Evidence for a metabolic partnership. *Fitoterapia*, *105*, 147–150. https://doi.org/10.1016/j.fitote.2015.06.018

Weggemans, R. M., Zock, P. L., & Katan, M. B. (2001). Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol in humans: a meta-analysis. *The American journal of clinical nutrition*, *73*(5), 885–891. https://doi.org/10.1093/ajcn/73.5.885