

Effects of coconut water and bitter leaf on Triglycerides and Cholesterol in Cyclosporine- induced Wistar Rats

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ABSTRACT

This study investigates the effects of aqueous extracts of *Vernonia amygdalina* and coconut water on Triglyceride and Cholesterol of cyclosporine-induced Wistar rats. By examining their impact on cholesterol and triglycerides, this research seeks to provide evidence for their potential therapeutic applications. Sixteen(16) Cyclosporine-induced cholesterol Albino wistar rats were divided into four (4) groups (Group A-D) consisting of four (4) rats in each group, which were tagged and housed in separate cages Group A served as the negative control group and will be administered sterile water and standard feed throughout the experiment. Group B served as the experimental group and will be administered 10mg/kg cyclosporine for the experiment. Group C served as the experimental group which is induced with 200mg/kg cyclosporine for hypercholesterol and will be administered 5ml/kg of the extract for the experiment. Group D served as the experimental group which is induced with 400mg/kg cyclosporine for hypercholesterol and were administered 10ml/kg of the extract for the experiment. Blood samples were collected between the hours of 8 and 11 in the morning on the same day. Triglyceride and Cholesterol, were all analyzed with the help of Blood Lipid Panel test. One-way analysis of variance (ANOVA) was used to evaluate the data, and the Statistical Package for the Social Sciences (SPSS) (International Business Machines (IBM), United States) Statistics v.23 package was used to analyze the results, with $p < 0.05$ being considered significantly. The data was expressed as mean $\pm$ standard error of mean. As demonstrated by the decreases in cholesterol and triglyceride levels, the current study demonstrated a substantial hypolipidemic effect of the aqueous extract of *Vernonia amygdalina* and Buko juice of cyclosporine-induced dyslipidemia in Wistar rats.

Keywords: *Vernonia amygdalina*, buko juice, cyclosporine, wistar rats, triglyceride, cholesterol

INTRODUCTION

The increasing global interest in plant-based treatments and natural remedies stems from their wide range of medicinal benefits, minimal side effects,[13,14] and high therapeutic potential.[11] Many plant

species worldwide possess bioactive compounds with significant pharmacological effects on the human body.[17] These plants are utilized in both herbal and conventional medicine, offering unique advantages over synthetic drugs,

such as enhanced support for the body's healing processes.[16] Vernonia amygdalina (commonly known as bitter leaf) and coconut water (locally termed Buko juice) are among these promising natural remedies.

Diabetes, a major contributor to global morbidity and mortality, has seen a dramatic rise in prevalence, with cases in the United States alone escalating from 5.8 million in 1980 to 94.7 million by 2004.[5] Various risk factors, including genetic predisposition, sedentary lifestyles, obesity, hyperinsulinemia,[18] and smoking[6,7] have been identified. The use of natural compounds such as those derived from Vernonia amygdalina, rich in saponins, alkaloids, flavonoids, and phenolic acids, has shown potential in addressing health issues such as gastrointestinal disorders, parasitic infections, and metabolic imbalances.

Coconut water, another natural product, is valued for its isotonic properties and rich composition, including potassium, magnesium, and vitamin B complexes like thiamine. Extracted from young coconuts, it provides hydration and supports physiological functions, making it a popular health-promoting drink.

Cyclosporine, a widely used immunosuppressive drug, is essential in organ transplantation and autoimmune disease management. However, its use is associated with adverse effects, particularly dyslipidemia, characterized by elevated cholesterol and triglycerides, which significantly increase the risk of cardiovascular diseases.[10] Dyslipidemia occurs due to cyclosporine's inhibition of bile acid synthesis and reduction in lipoprotein lipase activity, leading to abnormal lipid metabolism and high cholesterol levels.[8] This underscores the urgent need for alternative or

complementary treatments to mitigate these side effects.

Because medications are so expensive, more than 86% of people in developing nations rely on plant-based cures, making traditional medicine an essential resource (World Health Organization [WHO], 2004).[19] The WHO emphasizes the importance of research into plant-derived compounds to develop effective treatments for chronic conditions like hypercholesterolemia.[9,12] Although over 400 traditional plant remedies have been documented for therapeutic use, only a fraction have undergone rigorous scientific validation.[15]

This study investigates the effects of aqueous extracts of Vernonia amygdalina and coconut water on the lipid profiles of cyclosporine-induced albino Wistar rats. By examining their impact on key lipid markers, including total cholesterol (T-Chol) and triglycerides (Tg), this research seeks to provide evidence for their potential therapeutic applications. The findings aim to contribute to the growing body of knowledge on the use of natural remedies in managing metabolic disorders, offering insight into their mechanisms and paving the way for future clinical applications.

MATERIALS AND METHODS

The following materials were used in this study:

- Fresh mature leaves of Vernonia amygdalina
- Fresh Buko juice commercially available
- Wistar rat
- Cages
- Bedding
- Feed
- Hand Gloves
- Distilled water
- Heparinized/EDTA bottle
- Cyclosporine Drug

- Centrifuge
- Spectrophotometer

COLLECTION OF PLANT MATERIALS

Fresh *Vernonia amygdalina* plant leaves were obtained locally at the International Market in Abakaliki, Ebonyi State. The Buko jice plants were locally sourced from within Ahia-ofu, Ebonyi State, Abakaliki.

Preparation of the Aqueous Extract of *Vernonia amygdalina* Leaves

Fresh leaves of *Vernonia amygdalina* were first sorted to remove dry ones. They were rinsed and air-dried at room temperature by spreading them on a laboratory Table for 7 days. They were ground to a mesh size of 1mm and 400mg of powdered material was extracted.

Buko Juice from Green coconuts (*Cocos nucifera* Linnaeus) between 6 and 8 months of age used in this work, was extracted by perforation with a manual, stainless-steel punch after the primary epicarp had been brushed and washed with water. The total water from the 12 selected fruits was homogenized, filtered through a whatman filter paper of 1:11µm and maintained at -20 °C to prevent any enzymatic or microbiological action. To achieve a concentration of 20 mg/ml, 400 mg of the dry residue was dissolved in 20 ml of buko juice extract. The following concentrations were prepared from this stock solution: 5, 10, 20, 40 and 60 percent of aqueous solutions.

ANIMAL CARE AND EXPERIMENTAL DESIGN

Animal Care

Sixteen (16) Cyclosporine-induced cholesterol wistar rats within the weight range of 140g to 190g were used for this study. They were obtained from the animal house of the Department of Physiology, Faculty of Basic Medical Sciences,

Ebonyi State University, Abakaliki, Nigeria. The animals were housed in a cage to

Throughout this study, the animals were acclimated for seven days under a 12-hour light/dark cycle and were treated in compliance with the World Health Organization's (1998) good laboratory practice (GLP) guidelines and the National Institute of Environmental and Health Sciences' (NIEHS) 1985 animal care guidelines. The rats were given the regular diet and free access to clean water for the duration of the fourteen-day trial.

Experimental Grouping

Four (4) groups (Groups A–D) of sixteen (16) cyclosporine-induced cholesterol wistar rats were created, each with four (4) rats. The rats were tagged and kept in different cages.

1. Group A served as the negative control group and were administered sterile water and standard feed throughout the experiment.
2. Group B, the experimental group, received 10 mg/kg of cyclosporine.
3. Group C, the experimental group, received 5 ml/kg of the extract and was given 200 mg/kg of cyclosporine to treat hypercholesterolemia.
4. Group D, the experimental group, received 10 milliliters per kilogram of the extract and was given 400 milligrams per kilogram of cyclosporine to treat hypercholesterolemia.

ADMINISTRATION OF EXTRACT

Administration of extract is by oral route. The rat was held at the muscles behind the neck and back and then turned so the mouth faced upwards. The cannula was then inserted into the esophagus and its content was then gradually emptied while

Being careful not to enter the respiratory tract.

ACUTE TOXICITY TEST (MEDIUM LETHAL DOSE:LD50)

The method of Aniagueta^{al.}, (2005) was employed in the acute toxicity studies (LD50) of the extract. Eight (8) adult rats were used in this three (3) day LD50 study. On the first day, six (6) adult rats were used and divided into three (3) groups. Following that, they were given doses of the aqueous leaf extract of Bukojuice and Vernonia amygdalina that amounted to 10 mg/kg, 100 mg/kg, and 1000 mg/kg of body weight. They were all promptly observed for 30 minutes, 4 hours, and 24 hours. The rats were given 1600 mg/kgbw, 2900 mg/kgbw, and 5000 mg/kgbw on the second day. A control group of two rats was given 1 ml of distilled water on the third day. Suppression of appetite in these animals may have activated compensatory physiological responses to cope with the stress of hunger and thirst (Babayietal,2007) which may have led to reduction in feed and fluid consumption of the animals administered with 1000, 2000 and 3000mg/kgbw of the extract. However, the acute toxicity studies showed an LD50 value that was greater than 5000mg/kg of

Body weight.

COLLECTION OF BLOOD SAMPLES FOR LIPID PROFILE

Requirements include animal, aesthetic agent, cotton wool, capillary tube, and blood sample collection tubes. Blood sample was collected under general anesthesia from the superior and inferior venacava of the Cyclosporine-induced wistar rats. The blood samples were collected between the hours of 8 and 11 in the morning on the same day.

MEASUREMENT OF LIPID PROFILE

The determination of Lipid Profile: T-Chol, Tg HDL-C, LDL, were all analyzed with the help of Blood Lipid Panel test.

STATISTICAL ANALYSIS

The data was expressed as mean $\pm$ standard error of mean and statistically evaluated using one- way analysis of variance (ANOVA) followed by Statistical Package for the social sciences (SPSS) (International Business Machines (IBM), United States) Statistics v.23 package was used to analyze the results with $p < 0.05$, Considered significantly.

RESULTS

Table 1: Showing data collected from the experiment done on the animals.

	Mmol/l Chol	Mmol/l Tag	Mmol/l HDL	Mmol/lLDL
G1R1	4.8	1.7	1.4	3.06
G1R2	4.3	1.4	1.9	2.12
G2R1	5.1	1.5	1.7	3.10
G2R2	4.9	1.5	1.6	3.00
G3R1	5.0	1.6	1.5	3.18
G3R2	4.8	1.6	2.0	2.48
G4R1	4.6	1.5	1.8	2.50
G4R2	4.7	1.4	1.7	2.72

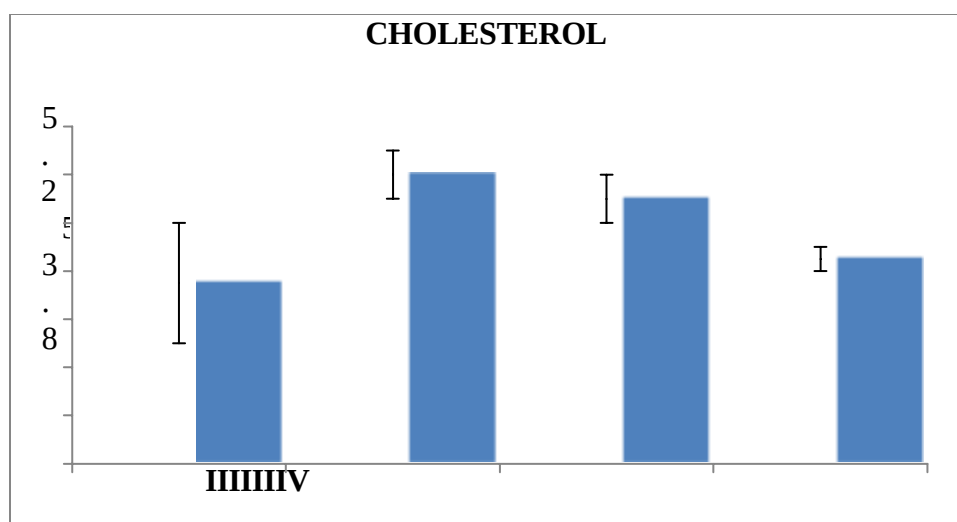


Fig. 1: Showing the Effect of aqueous extract of *Vernonia Amygdalina* and Buko Juice On Cholesterol level in wistar rats N=12.

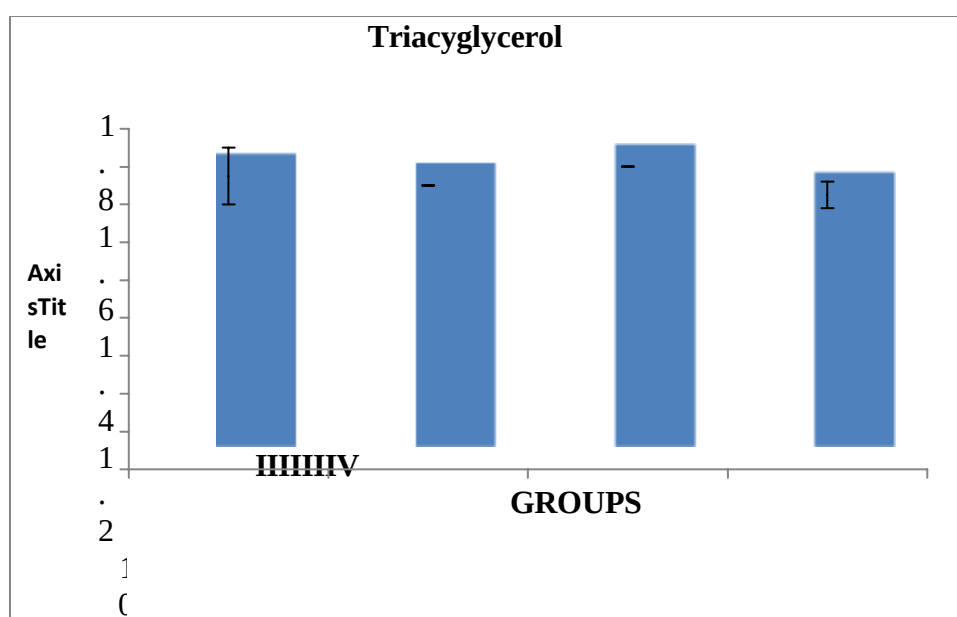


Fig. 2: Effect of aqueous extract of *Vernonia Amygdalina* and Buko Juice on Triacylglycerol Level in wistar rats.

DISCUSSION

This study aimed to investigate the effects of the aqueous extract of *Vernonia amygdalina* and Buko juice on the lipid profile of cyclosporine-induced dyslipidemia in Wistar rats. The results revealed a significant hypolipidemic effect of these treatments, as indicated by the reductions in total cholesterol (TC) and triglyceride (TG) levels. This finding is consistent with earlier studies reporting the

lipid-lowering and antioxidative properties of *Vernonia amygdalina* in animal.[1,4] Moreover, the observed reduction in body weight following treatment supports the hypothesis that these plants may improve insulin sensitivity, as weight loss is often associated with enhanced insulin-stimulated reduction in lipoprotein lipase (LPL) activity, leading to lower lipid content in adipose tissue.[2]

The cyclosporine-induced group (G2) exhibited elevated levels of cholesterol (5.1 and 4.9 mmol/L) compared to the control group, which had cholesterol levels of 4.8 and 4.3 mmol/L. Triglyceride levels were also slightly higher in the cyclosporine group (1.5 mmol/L) compared to the control (1.7 and 1.4 mmol/L). Treatment with *Vernonia amygdalina* (G3) and Buko juice (G4) led to a significant reduction in cholesterol levels, suggesting that both treatments can mitigate the dyslipidemic effects of cyclosporine. However, triglyceride levels remained close to the control values, indicating that these treatments did not significantly affect triglycerides, a finding that warrants further investigation. These observations are in line with previous studies that have demonstrated the efficacy of herbal treatments in modulating lipid profiles (Nwanjo, 2005; Omeniyi et al., 1996).[3]

The combination of *Vernonia amygdalina* and Buko juice produced the most substantial improvements in lipid parameters, suggesting a potential synergistic effect. The lipid profiles of the treated groups were closer to those of the control group than to the cyclosporine-treated group, supporting the therapeutic potential of these natural agents in managing cyclosporine-induced dyslipidemia. This is consistent with the findings of Akah and Okafor (2008), who reported the significant lipid-lowering effects of *Vernonia amygdalina* in animal models, which were also evident in the present study. Furthermore, the significant improvements observed in the treated groups align with the observed effects of other plant-based therapies for managing dyslipidemia (Omeniyi et al., 1996). These findings suggest that *Vernonia amygdalina* and Buko juice may provide a beneficial adjunctive treatment for patients undergoing cyclosporine therapy.

This study demonstrated that both the aqueous extract of *Vernonia amygdalina* and Buko juice significantly mitigated the dyslipidemic effects induced by cyclosporine in albino Wistar rats. The combination of these two treatments yielded the most pronounced improvements in lipid profiles, indicating a potential synergistic effect. These results imply that Buko juice and *Vernonia amygdalina* may be useful natural remedies for cyclosporine-induced dyslipidemia. Given the promising results in this animal model, further clinical research is essential to validate these findings in humans. The integration of such therapies could offer a holistic approach to improving the health and quality of life of patients undergoing cyclosporine therapy. Previous studies have similarly suggested the value of combining herbal treatments for improving lipid profiles in clinical settings (Akah & Okafor, 2008; Nwanjo, 2005).

Although the results in Wistar rats are promising, human studies are necessary to confirm the efficacy and safety of *Vernonia amygdalina* and Buko juice. Clinical trials should involve diverse populations, assessing various dosages to determine the most effective therapeutic protocols for humans. This aligns with the recommendations of Akah and Okafor (2008), who emphasized the importance of translating animal model findings into human clinical trials. Future research should focus on identifying and isolating the bioactive compounds responsible for the observed lipid-lowering effects of *Vernonia amygdalina* and Buko juice. Understanding the mechanisms of action can guide the development of more targeted and effective therapies for dyslipidemia and related conditions (Defronzo & Ferannjimi, 1991). Long-term studies are essential to evaluate the sustained effects of *Vernonia amygdalina* and Buko juice on lipid profiles and

overall cardiovascular health. These studies should also examine any potential side effects and interactions with other medications, especially in chronic use. Dose-response studies are crucial for determining the optimal dosages for both *Vernonia amygdalina* extract and Buko juice, ensuring maximum therapeutic benefits with minimal adverse effects (Omeniyi et al., 1996). Further investigations should explore the potential benefits of *Vernonia amygdalina* and Buko juice in managing other conditions associated with dyslipidemia and oxidative stress, such as diabetes, obesity, and metabolic syndrome (Nwanjo, 2005).

CONCLUSION

This present study revealed a significant hypolipidemic effect of aqueous extract of *Vernonia amygdalina* and Buko juice of cyclosporine-induced dyslipidemia in Wistar rats, as indicated by the reductions in cholesterol and triglyceride levels.

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