

Effects of Aqueous extract of Buko Juice (coconut water) and Amygdalina (bitter leaf) on lipid Profile in Cyclosporine- induced Wistar Rats

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ABSTRACT

Vernonia amygdalina, commonly known as bitter leaf, is a shrub that thrives in the African tropics, particularly in Nigeria, Cameroon, and Zimbabwe. It is known for its wide range of health benefits, including antimicrobial, antiparasitic, and gastrointestinal relief properties. Its organic extracts contain biologically active compounds such as saponins, alkaloids, flavonoids, and phenolic acids, which contribute to its medicinal potential. Buko juice, or coconut water, is another natural product with therapeutic benefits, offering electrolytes like potassium and magnesium along with essential vitamins. Traditionally, the liquid from young coconuts is favored for its refreshing and nourishing properties. Cyclosporine, an immunosuppressant commonly used in organ transplantation and autoimmune disorders, has been associated with adverse effects such as dyslipidemia, characterized by elevated cholesterol and triglyceride levels. This condition increases the risk of cardiovascular diseases, necessitating the search for alternative or complementary therapies to mitigate these side effects (Kasiske et al., 2001). Given the potential of natural products like *Vernonia amygdalina* and Buko juice, exploring their effects on lipid metabolism could provide valuable insights into managing hypercholesterolemia, particularly in patients undergoing cyclosporine therapy. 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 hypercholesterolemia 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 hypercholesterolemia and will be 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. Lipid Profile: T-Chol, Tg, HDL-C, LDL, were all analyzed with the help of Blood Lipid Panel test. Data obtained 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. This study highlights the potential of Vernonia amygdalina and Buko juice as natural remedies for managing cholesterol levels, particularly in patients undergoing cyclosporine therapy. There was significant reductions in total cholesterol and improvements in HDL levels suggest that these extracts could serve as complementary therapies alongside conventional treatments.

Key Words: *Vernonia amygdalina (bitter leaf), Buko juice (coconut water), total cholesterol, HDL and Wistar Rat.*

INTRODUCTION

In recent years, the search for substances or agents with medicinal value has increasingly turned toward plants and plant-based products. This shift is driven by the perception that plant-derived compounds generally have fewer side effects compared to synthetic medications (Prout, 1974) and possess rich medicinal properties (Obi et al., 1988).

Many plant species worldwide contain active constituents that can directly influence physiological processes (Uahomo et al., 2022). These plants play significant roles in both herbal and conventional medicine, providing benefits that are often absent in pharmaceuticals, such as enhancing the body's ability to combat illness and restore health (Singh et al., 2007).

Vernonia amygdalina, commonly known as bitter leaf, is a shrub that thrives in the African tropics, particularly in Nigeria, Cameroon, and Zimbabwe. It is known for its wide range of health benefits, including antimicrobial, antiparasitic, and gastrointestinal relief properties. Its organic extracts contain biologically active compounds such as saponins, alkaloids, flavonoids, and phenolic acids, which contribute to its medicinal potential. Buko juice, or coconut water, is another natural product with therapeutic benefits, offering electrolytes like potassium and magnesium along with essential vitamins. Traditionally, the liquid from young

coconuts is favored for its refreshing and nourishing properties.

Cyclosporine, an immunosuppressant commonly used in organ transplantation and autoimmune disorders, has been associated with adverse effects such as dyslipidemia, characterized by elevated cholesterol and triglyceride levels. This condition increases the risk of cardiovascular diseases, necessitating the search for alternative or complementary therapies to mitigate these side effects (Kasiske et al., 2001). Given the potential of natural products like Vernonia amygdalina and Buko juice, exploring their effects on lipid metabolism could provide valuable insights into managing hypercholesterolemia, particularly in patients undergoing cyclosporine therapy.

The current research aims to investigate the effects of aqueous extracts of Vernonia amygdalina (bitter leaf) and Buko juice (coconut water) on the lipid profile of cyclosporine-induced albino Wistar rats. This study seeks to determine their impact on key lipid markers, including cholesterol and triglycerides, through controlled experimentation. Understanding the potential of these natural extracts could contribute to the development of alternative strategies for managing lipid imbalances associated with immunosuppressive therapy, paving the way for future research and application in clinical settings.

MATERIALS AND METHODS

The following are materials to be used in this study:

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

COLLECTION OF PLANT MATERIALS

The Fresh leaves of *Vernonia amygdalina* plants were locally sourced from within international market, Ebonyi State, Abakaliki.

The *Buko juice* 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 400 mg 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 1:11 μ m and

maintained at -20°C to prevent any microbial or enzymatic activity.

400mg of the dried residue was dissolved in 20 ml of Buko juice extract to give a concentration of 20mg/ml. The following concentrations were then prepared from this stock solution: 5, 10, 20, 40 and 60 percent of aqueous solutions.

ANIMAL CARE AND EXPERIMENTAL DESIGN

Animal care

Twelve (12) 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, Abakiliki, Nigeria. The animals were housed in a cage to acclimatize for seven days under a 12-hour light/dark cycle and cared for in accordance with good laboratory practice (GLP) regulations of World Health Organisation (1998) and the principles of animal care (National Institute of Environmental and Health Sciences (NIEHS), 1985) were followed throughout this study. The experiment commenced and lasted for fourteen days, with the rats being fed the standard feed and clean water ad libitum.

Experimental Grouping

The 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 was administered sterile water and standard feed throughout the experiment.
- Group B served as the experimental group and was administered 10mg/kg cyclosporine for the experiment.

- iii. Group C served as the experimental group which is induced with 200mg/kg cyclosporine for hypercholesterolemia and was administered 5ml/kg of the extract for the experiment.
- iv. Group D served as the experimental group which is induced with 400mg/kg cyclosporine for hypercholesterolemia and was administered 10ml/kg of the extract for the experiment.

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 Aniaguet *et 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. They were then treated with doses of the aqueous leaf extract of *vernoniaamygdalina* and *Buko juice* corresponding to 10 mg/kg, 100 mg/kg and 1000mg/kg of body weight and were all placed under observation immediately for thirty(30)minutes, four (4) hours and twenty-four (24) hours. On the second day, the rats were treated with 1600 mg/kgbw and 2900 mg/kgbw; and 5000 mg/kgbw and on the third day, a

group of two rats served as control, receiving 1 ml of distilled water.

Suppression of appetite in these animals may have activated compensatory physiological responses to cope with the stress of hunger and thirst (Babayiet *al*, 2007) which may have led to reduction in feed and fluid consumption of the animals administered with 1000, 2000 and 3000 mg/kgbw of the extract. However, the acute toxicity studies showed an LD₅₀ value that was greater than 5000mg/kg of bodyweight.

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 vena cava 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 4.1: *Effect of aqueous extract of Vernonia Amygdalina and Buko Juiceon Total Cholesterol Lipid profile*

DOSAGE TYPE	ANIMAL GROUPING	T-CHOL VALUE (mg/dl)	T-CHOL VALUE (Mmol/L)	MEAN+- SEM
-Ve Control (Group A)	Rat 1	190	4.91	190+_
10mg/kg of cyclosporine (Group B)	Rat 2	210	5.4	
10mg/kg of cyclosporine and 200ml/kg of extract (Group C)	Rat 3	195	5.04	
10mg/kg of cyclosporine and 400ml/kg of extract(Group D)	Rat 4	165	4.2	

Results=Mean±SD. *P<0.05 compared with control.

This study observed significant changes in cholesterol levels among the different treatment groups. As shown in Table 4. 1, the total cholesterol levels for Group A, Group B, Group C, and Group D were 190, 210, 195, and 165 mg/dL, respectively. The data revealed that

cyclosporine induction in Group B led to a borderline increase in cholesterol levels when compared to the negative control. However, subsequent treatment with Vernonia amygdalina and Buko juice resulted in a significant ($P < 0.05$) decrease in total cholesterol across Groups C and D.

Table 4.2: *Effect of aqueous extract of Vernonia Amygdalina and Buko Juiceon HDL Lipid Profile*

DOSAGE TYPE	ANIMAL GROUPING	HDL VALUE (mg/dl)	HDL VALUE (Mmol/L)	MEAN+- SEM
-Ve Control (Group A)	Rat 1	60	1.55	47+_
10mg/kg of cyclosporine (Group B)	Rat 2	30	0.7	
10mg/kg of cyclosporine and 200ml/kg of extract (Group C)	Rat 3	40	1.03	
10mg/kg of cyclosporine and 400ml/kg of extract(Group D)	Rat 4	58	1.49	

Results=Mean±SD. *P<0.05 compared with control.

In this study, the administration of aqueous extracts of Vernonia amygdalina and Buko juice positively influenced HDL levels in cyclosporine-induced Wistar rats. As shown in Table 2, the mean HDL levels for Groups A, B, C, and D were 60, 30, 40, and 58 mg/dL, respectively. Following cyclosporine induction, Group B experienced a notable decrease in HDL levels compared to the negative control

group, indicating an adverse effect of cyclosporine on HDL. However, treatment with vernoniaamygdalina and Buko juice led to significant ($P < 0.05$) improvements in HDL levels. Group C saw a 25% increase in HDL levels with a 100 mg/kg dose over two days, while Group D showed a 31% increase with a 400 mg/kg dose over five days.

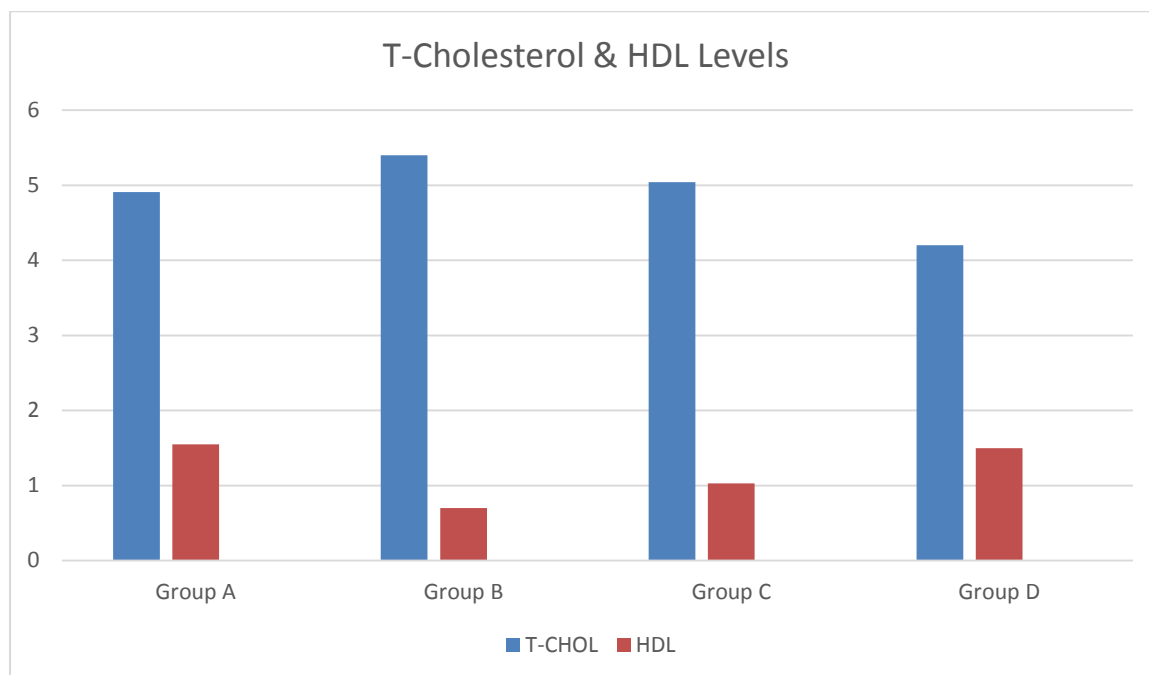


Fig. 4.1: Effect of aqueous extract of *Vernonia Amygdalina* and Buko Juice on Total

Cholesterol and HDL Lipid profile levels.

As shown in Fig. 4. 1, the total cholesterol levels for Group A, Group B, Group C, and Group D were 190, 210, 195, and 165 mg/dL, respectively. The data revealed that cyclosporine induction in Group B led to a borderline increase in cholesterol levels when compared to the negative control. However, subsequent treatment with *Vernonia amygdalina* and Buko juice resulted in a significant ($P < 0.05$) decrease in total cholesterol across Groups C and D.

As shown in Fig.4 1, the mean HDL levels for Groups A, B, C, and D were 60, 30, 40, and 58 mg/dL, respectively. Following cyclosporine induction, Group B experienced a notable decrease in HDL levels compared to the negative control group, indicating an adverse effect of cyclosporine on HDL. However, treatment with *vernoniaamygdalina* and Buko juice led to significant ($P < 0.05$) improvements in HDL levels. Group C saw a 25% increase in HDL levels with a 100 mg/kg dose over two days, while Group D

showed a 31% increase with a 400 mg/kg dose over five days.

DISCUSSION

This study explored the effects of aqueous extracts of **Vernonia amygdalina** and Buko juice on lipid profiles, focusing on total cholesterol (T-Chol) and high-density lipoprotein (HDL) levels in cyclosporine-induced albino Wistar rats. The findings provide insights into the potential hypolipidemic properties of these extracts, aligning with previous research on the medicinal value of plant-based treatments.

Effect on Total Cholesterol (T-Chol)

Hypercholesterolemia, characterized by elevated cholesterol levels, can be caused by genetic factors, lifestyle choices, underlying health conditions, or certain medications like cyclosporine (Sergio & Juan, 2019). This study observed significant changes in cholesterol levels among the different treatment groups. As shown in Table 1, the total cholesterol levels for Group A, Group B, Group C, and Group D were 190, 210, 195, and 165 mg/dL, respectively. The data revealed

that cyclosporine induction in Group B led to a borderline increase in cholesterol levels when compared to the negative control. However, subsequent treatment with *Vernonia amygdalina* and Buko juice resulted in a significant ($P < 0.05$) decrease in total cholesterol across Groups C and D.

The most substantial reduction in cholesterol was observed in Group D, where a 400 mg/kg dose of the extracts was administered for five days, leading to a 15.4% decrease. Meanwhile, Group C, which received a 200 mg/kg dose over two days, exhibited a 7.5% reduction. This suggests that a higher dose and prolonged administration may amplify the cholesterol-lowering effects of the extracts. These results support the hypolipidemic properties of *Vernonia amygdalina*, consistent with previous studies, such as Olabisi Olafunmilayo Ogunrinola et al. (2019), which demonstrated significant reductions in cholesterol levels in cadmium-induced rats. Similarly, Gospel Ajuru et al. (2003) and Akah & Okafor (2008) reported comparable hypolipidemic effects in Wistar rats, attributing the effects to the bioactive compounds present in *Vernonia amygdalina*.

Effect on HDL Cholesterol

LDL cholesterol is an important predictor of cardiovascular health, with low levels (hypoalphalipoproteinemia) being a risk factor for coronary heart diseases and atherosclerosis (Raster & Degoma, 2012). In this study, the administration of aqueous extracts of *Vernonia amygdalina* and Buko juice positively influenced HDL levels in cyclosporine-induced Wistar rats. As shown in Table 4. 2, the mean HDL levels for Groups A, B, C, and D were 60, 30, 40, and 58 mg/dL, respectively. Following cyclosporine induction, Group B experienced a notable decrease in HDL levels compared to the negative control

group, indicating an adverse effect of cyclosporine on HDL.

However, treatment with *vernonia amygdalina* and Buko juice led to significant ($P < 0.05$) improvements in HDL levels. Group C saw a 25% increase in HDL levels with a 100 mg/kg dose over two days, while Group D showed a 31% increase with a 400 mg/kg dose over five days. These findings align with the results of Olabisi et al. (2019), where similar increases in HDL were noted in rats treated with *Vernonia amygdalina*. Moreover, Mensik et al. (2003) observed that the polyunsaturated fatty acids in *Vernonia amygdalina* could contribute to elevated HDL levels, further supporting the findings of this study.

Limitations of the Study

Despite the promising results, the study faced some limitations. The use of animal models restricts the generalizability of findings to human subjects, and further research is needed to confirm these effects in clinical settings. Additionally, the study focused on a short duration of treatment; longer-term studies could provide more comprehensive insights into the sustained effects of these extracts on lipid profiles.

Conclusion

This study highlights the potential of *Vernonia amygdalina* and Buko juice as natural remedies for managing cholesterol levels, particularly in patients undergoing cyclosporine therapy. The significant reductions in total cholesterol and improvements in HDL levels suggest that these extracts could serve as complementary therapies alongside conventional treatments. Future studies should explore the underlying mechanisms of these effects and consider clinical trials to evaluate their applicability in human populations. Expanding the understanding of these natural extracts may pave the way for safer, cost-effective interventions for

hyperlipidemia and related cardiovascular conditions.

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