



COMPARATIVE ANALYSIS OF THE EFFECTS OF THE AFRICAN WALNUT (TETRACARPIDIUM CONOPHRUM) AND DOUBLE ROOT COFFEE ON LIBIDO, HORMONAL PROFILE (TESTOSTERONE) AND LIVER FUNCTION ON ADULT MALE WISTAR RATS

Ezeokafor Emmanuel Nonso¹, Ugochukwu Precious Chisom², Ebisintei Precious³, Ezeagu Darlington Kenekchukwu⁴, Sebastine Okechukwu Nwoko⁵, Afubero Francis Chukwudi⁶, Okoye Ogochukwu Fidelis⁷, Charles Chinemeze Anyaogu⁸, Ejeh Evelyn Ene^{9*}

^{1,2,6,8,9}Department of Human Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus.

⁴Department of Human Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus.

⁵Department of Human Biochemistry, Nnamdi Azikiwe University, Nnewi Campus.

⁷Department of Human Physiology, Faculty of Basic Medical Sciences, Chukwu Ojukwu Odumegwu University, Uli.

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***Corresponding Author**

Ejeh Evelyn Ene

Department of Human
Physiology, Faculty of Basic
Medical Sciences, Nnamdi
Azikiwe University, Nnewi
Campus.

ABSTRACT

The consumption of double-root coffee and African walnut (*Tetracarpidium conophorum*) has garnered attention for their potential effects on libido and sexual drive. Double-root coffee, known for its stimulant properties, contains caffeine and other bioactive compounds that may enhance sexual arousal by improving blood circulation and energy levels. Conversely, African walnuts are rich in omega-3 fatty acids, antioxidants, and essential nutrients contributing to overall reproductive health. Research indicates that consumption of walnuts may improve sperm quality and vitality, thereby positively influencing male fertility. Furthermore, these foods' neuroprotective and anti-inflammatory properties can mitigate oxidative stress in reproductive tissues, which is often linked to decreased libido. This abstract aims to explore the synergistic effects of these two dietary components on liver function and sexual health by reviewing existing literature on their

nutritional profiles and biological impacts on libido enhancement. Twenty male rats and eight female rats weighing 192-257g were divided into five groups of four animals each. Group 1 served as the control group which received just feed and water. Group 2 had four rats and they were administered with just walnuts, Group 3 had the same but was administered with double-root coffee. Group 4 had four animals also and they were fed with the combination of walnut and double-root coffee but at a low dose, Group 5 had the same but at a higher dose. This experiment lasted for a total of two weeks and the results showed that there was a significant increase in testosterone levels, a decrease in body weight, and normal levels of liver function which means there was no damage to the liver.

KEYWORDS: walnut, double root coffee, wistar rats.

1.0 INTRODUCTION

African walnut (*Tetracarpidium conophorum*) and double root coffee are two dietary components traditionally consumed in West Africa and Asia, respectively, for their health-promoting properties. African walnuts are rich in omega-3 fatty acids, zinc, phenolic compounds, and antioxidants, all of which have been linked to improved reproductive health and hepatoprotection. Studies have shown that walnut supplementation enhances testosterone synthesis, sperm motility, and sexual behaviour in animal models by reducing oxidative stress in Leydig cells and increasing nitric oxide bioavailability, which facilitates erectile function (Akomolafe & Oboh, 2017).

Double root coffee, a formulation of coffee combined with adaptogenic herbs such as Maca (*Lepidium meyenii*) and Tongkat Ali (*Eurycoma longifolia*), is reputed to improve libido, sexual stamina, and hormonal balance. Caffeine, the principal stimulant, has been shown to enhance central nervous system activity, improve energy metabolism, and promote sexual arousal. Tongkat Ali reduces sex hormone-binding globulin (SHBG), increasing free testosterone, while Maca regulates the hypothalamic-pituitary-gonadal axis to promote androgen production (Tambi et al., 2012).

Despite the popularity of these natural products, limited studies have examined their combined effects on reproductive hormones and liver function, particularly in controlled animal models. Since liver integrity is critical for hormonal metabolism, it is essential to evaluate the hepatoprotective or hepatotoxic potential of these agents when used singly or in combination. This study therefore investigates the effects of African walnut and double root

coffee on serum testosterone levels and liver function markers (AST, ALT, and ALP) in Wistar rats, with a view to determining their potential therapeutic benefits and safety profile.

2.0 MATERIALS AND METHODS

2.1 Ethical approval

The ethical approval was obtained from the Faculty of Basic Medical Science ethical committee, Faculty of Basic Medical Science, College of Health Science, Nnamdi Azikiwe University, Nnewi Campus.

2.2 LOCATION OF THE STUDY

This study was conducted in the Animal House, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

2.3 RESEARCH DESIGN

An experimental and descriptive design with a random controlled trial was employed in this study.

2.4 MATERIALS

This research was carried out using the following materials:

20 Male Wistar rats.

Latex medical hand gloves.

Standard cages.

Rat feed(Top feeds finisher).

Animal weighing balance Camry 11LB China.

Electronic weighing balance XY3000 China.

Centrifuge model PLC 03 Taiwan.

Measuring cylinder.

Beaker.

Thermostat Oven DHG 9023A PEC Medicals USA.

Digital Rotary Evaporator TT-55 Techmel & Techmel USA.

Absolute Ethanol (BDH England).

Light Microscope (Olympus) XSZ-107BX.

Oral cannula.

2ml, 4ml, and 5ml syringes (Disposable).

Watch.

Camera.

Walnut extract.

Double-root coffee.

Distilled water.

EDTA bottle.

Refrigerator (Nexus).

METHODOLOGY

2.5 EXTRACTION PROCEDURE

Fresh seed of Walnuts was purchased from the Nkwo Local market in Nnewi, Nnewi North local government area of Anambra state.

The seed was cracked open and sliced. The sliced seeds were dried using a Thermostat Oven (DHG-9023A PEC Medicals USA).

The dried seed was grounded using a local grinder into a coarse form. 200g of the grounded seed was macerated in 1000mls of 98% absolute ethanol (BDH England) for 48 hours.

The mixture was sieved after 48 hours with porcelain cloth into a clean glass beaker. The filtrate was concentrated using a Digital Rotary Evaporator (TT-55 Techmel & Techmel, USA) and was further dried using a thermostat oven into a paste-like form and was preserved in a refrigerator (Nexus).

The procedure was carried out according to the method described by Al Atter, 2012.

2.6 PLANT PREPARATION

The walnuts were obtained from the Nkwo market, in Nnewi, close to the College of Health Sciences, Nnamdi Azikiwe University, Nnewi. The walnuts were sliced thinly and then left to dry. They were dried using a laboratory oven and also air-dried for one week.

After drying, the leaves were ground into a paste and stored in a refrigerator to avoid spoiling while in use.

2.7 EXPERIMENTAL ANIMALS

Twenty healthy Wistar rats weighing between 157-257g were obtained from the Animal House, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

The animals were grouped into five groups of four animals in each cage and kept under observation.

The animals were constantly fed with normal animal feed(finisher) and clean water. They were acclimatized for fourteen days.

2.8 EXPERIMENTAL DESIGN

The rats were weighed before the extract administration commenced and were weighed after the administration timeline was done.

The administration of the extracts were given as follows:

First, the animals were divided into five groups of two males and two females each aside from the control group which only had male rats.

Group 1 received just animal feed and water.

Group 2 received 200mg/kg of walnut=0.6ml dose only.

Group 3 received 200mg/kg of double root coffee=3.9ml dose only.

Group 4 received 200mg/kg of walnut and 200mg/kg of double root coffee= 0.4ml of walnut and 3.2ml of double-root coffee.

Group 5 received 200mg/kg of walnut and 200mg/kg of double root coffee= 0.8ml of walnut and 6.4ml of double-root coffee.

The administration was done through the oral route using an oral cannula and was administered once daily for 21 days (three weeks).

2.9 SAMPLE COLLECTION AND TERMINATION OF EXPERIMENT

At the end of the administration, the animals in their different groups were euthanized with mild anesthesia using chloroform in an enclosed container.

The blood was collected from each animal using a heparinized capillary tube following ocular puncture as detailed by Raveendran, Kesavan, and Parasuraman (2010) into a plain

tube. The blood that was collected was allowed to clot for a few minutes before it was centrifuged for 20 minutes at 3000 revolutions per minute, after that the serum was gotten using a micropipette.

Estimation of Liver enzymes

Alkaline Phosphatase (ALP) Estimation This test was carried out according to the method described by (King and Jegatheesan, 1959).

Principle: Alkaline Phosphatase in Alkaline medium hydrolyses Phenyl phosphatase in 15minutes at 37°C and pH of 10 to release phenol which in the presence of potassium ferricyanide reacts with 4-aminophenazone to give a red-pink colour which is measured Spectrophotometrically at 510nm wavelength. The intensity of the colour indicates ALP activity in the sample.

Aspartate Amino Transferase (AST) Estimation

This test was carried out according to the method described by (Obonga et al., 2019; Reitman and Frankel, 1957).

Principle: The substrate in the reaction are alpha ketoglutaric acid and L- Aspartate. The products formed by enzyme action are glutamate and oxaloacetate. Addition of 2, 4 dinitrophenyl hydrazine results in the formation of hydrazine complex with ketoacids. A red colour is produced on the addition of sodium hydroxide. The intensity of colour is related to the enzymatic activity and this can be measured at 550 nm wavelength using Spectrophotometer.

Alanine Amino Transferase (ALT) Estimation

This test was carried out according to the method described by (Reitman and Frankel, 1957).

Principle: The substrate in the reaction is alpha ketoglutaric acid and L- Aspartate. The products formed by enzyme action are Glutamate and Pyruvate. Addition of 2, 4 dinitrophenyl hydrazine results in the formation of hydrazine complex with ketoacids. A red colour is produced on the addition of sodium hydroxide. The intensity of colour is related to the enzymatic activity and this can be measured at 550 nm wavelength using Spectrophotometer.

3.0 Statistical Analysis

Data obtained in the study was subjected to Graph Pad Prism 9.5.1. Liver enzymes (AST, ALT, and ALP) activities and relative liver weight were analysed using ANOVA followed by post Hoc Fisher's LSD multiple comparison. Body weight was analysed using paired t-test and values were considered significant at $p \leq 0.05$.

RESULTS

Result presentation

Table 3.1: effect of ethanolic extract of walnut and double root coffee on body weight.

Group	Initial weight (g) MEAN $\pm$ SEM	Final weight(g) MEAN $\pm$ SEM	T-VALUE	P-VALUE
Group A (control)	118.67 $\pm$ 3.18	198.20 $\pm$ 5.01	-9.729	0.010
Group B (0.6ml walnut)	238.50 $\pm$ 3.18	233.50 $\pm$ 4.91	2.887	0.102
Group C (3.9ml of double-root coffee)	234.15 $\pm$ 3.55	224.95 $\pm$ 4.13	1.198	0.354
Group D (0.4ml of walnut and 3.2ml of double-root coffee)	243.35 $\pm$ 8.17	217.80 $\pm$ 6.41	1.753	0.222
Group E(0.8ml of walnut and 6.4ml of double-root coffee)	231.10 $\pm$ 5.14	215.45 $\pm$ 5.63	31.890	0.001

Data was analyzed using paired t-test and values were considered significant at $p \leq 0.05$, *: significant, #: not significant.

Table 1 result revealed an increase in the mean body weight in Group A, groups B, C, D, and E had a decrease in the body weight when the initial weight was compared to the final weight, which had a significant difference in Groups A and E, groups B, C, and D had no significant difference.

Table 3.2: effect of ethanolic extract of walnut and double root coffee on relative liver weight and relative testicular weight.

Group	Relative liver weight(g) MEAN±SEM	Relative testicular weight(g) MEAN±SEM
Group A (control)	4.14±0.15	0.67±0.01
Group B (0.6ml walnut)	3.37±0.05*	0.63±0.01#
Group C (3.9ml of double-root coffee)	3.07±0.03*	0.68±0.00#
Group D (0.4ml of walnut and 3.2ml of double-root) coffee	3.04±0.02*	0.70±0.02#
Group E (0.8ml of walnut and 6.4ml of double-root) coffee	3.21±0.08*	0.68±0.06#
P-value	31.238	1.137
F-ratio	0.001	0.393

Data was analyzed using Graph Pad Prism 9.5.1 using ANOVA followed by post-hoc LSD multiple comparisons. *: significant, #: not significant compared to group A.

Table 2 results showed a significant decrease in the mean relative liver weight in groups B, C, D, and E ($p=0.001$, $p=0.001$, $p=0.001$, $p=0.001$) compared to group A.

The mean relative testicular weight showed a decrease in group B, and groups C, D, and E had an increase compared to group A, which had no significant difference in groups B, C, D, and E ($p=0.247$, $p=0.805$, $p=0.440$, $p=0.821$).

Table 3.3: effect of ethanolic extract of walnut and double root coffee on serum AST, ALT, and ALP level.

Group	AST level(U/I) MEAN±SEM	ALT level(U/I) MEAN±SEM	ALP level (U/I) MEAN±SEM
Group A (control)	53.00±1.00	30.00±1.16	109.8±0.26
Group B (0.6ml walnut)	39.33±0.88*	25.00±0.58*	96.25±0.58*
Group C (3.9ml of double-root coffee)	40.67±0.88*	27.00±0.58*	98.78±0.81*
Group D (0.4ml of walnut and 3.2ml of double-root) coffee	42.00±0.58*	26.00±0.58*	98.03±0.59*
Group E (0.8ml of walnut and 6.4ml of double-root) coffee	47.00±0.58*	24.00±0.58*	104.00±2.13*
P-value	0.001	0.002	0.001
F-ratio	48.91	9.938	25.54

Data was analyzed using Graph Pad Prism 9.5.1 using ANOVA followed by post-hoc LSD multiple comparisons. *: significant, #: not significant compared to group A.

Table 3 results revealed a significant decrease in the mean AST level in groups B, C, D, and E ($p=0.001$, $p=0.001$, $p=0.001$, $p=0.001$) compared to A. Also, the mean ALT result showed a significant reduction in groups B, C, D, and E ($p=0.001$, $p=0.016$, $p=0.003$, $p=0.001$) compared to group A. Further, the mean ALP result revealed a significant decline in groups B, C, D, and E compared to group A.

Table 3.4: effect of ethanolic extract of walnut and double root coffee on testosterone level.

Group	Testosterone level(ng/ml) MEAN $\pm$ SEM
Group A (control)	0.41 $\pm$ 0.09
Group B (0.6ml walnut)	1.30 $\pm$ 0.03*
Group C (3.9ml of double-root coffee)	1.14 $\pm$ 0.01*
Group D (0.4ml of walnut and 3.2ml of double-root coffee)	0.97 $\pm$ 0.01*
Group E (0.8ml of walnut and 6.4ml of double root coffee)	0.93 $\pm$ 0.02*
P-value	0.001
F-ratio	9.724

5.0 DISCUSSION

African walnuts (*Tetracarpidium conophorum*) and double root coffee (a blend of coffee with adaptogenic herbs like Maca and Tongkat Ali) are increasingly recognized for their potential to enhance sexual health and hormonal balance. The African walnuts and double-root coffee helps in the enhancement of Testosterone Levels. African walnuts are rich in phenolic compounds, zinc, and omega-3 fatty acids, which contribute to their androgenic effects. A study by Akomolafe & Oboh (2017) demonstrated that walnut leaf extract significantly increased testosterone and luteinizing hormone (LH) levels in male rats, enhancing spermatogenesis and sperm motility. Similarly, a 2019 study found that African walnut seeds inhibited enzymes like phosphodiesterase-5 (PDE-5), which is critical for erectile function, while also increasing nitric oxide bioavailability—a key mediator of penile vasodilation (Ademiluyi et al., 2019). These effects are attributed to the antioxidant properties of phenolic compounds, which reduce oxidative stress in Leydig cells, thereby supporting testosterone synthesis (Ojobor et al., 2015). While double root coffee combines caffeine with adaptogens like Maca (*Lepidium meyenii*) and Tongkat Ali (*Eurycoma longifolia*). Maca root is known

to enhance libido and testosterone production via its effects on hypothalamic-pituitary-gonadal axis regulation. A 2025 clinical review highlighted that daily consumption of Maca-enriched coffee increased serum testosterone by 15–20% in men with mild hypogonadism (Zuma Coffee, 2025). Tongkat Ali, another key ingredient, has been shown to elevate free testosterone levels by reducing sex hormone-binding globulin (SHBG) activity, thereby increasing bioavailable testosterone (Tambi et al., 2012). They also help in increasing libido levels and sexual drive. A 2017 study on male rats fed walnut-supplemented diets reported a 40% increase in mounting frequency and reduced intromission latency, indicating heightened sexual motivation (Obboh et al., 2017). The researchers attributed this to walnut-derived L-arginine, a precursor to nitric oxide, which improves blood flow to erectile tissues. Additionally, the anxiolytic effects of walnut antioxidants reduced stress-related sexual dysfunction (Pulse Nigeria, 2024). Caffeine in coffee enhances libido by stimulating the central nervous system and increasing dopamine release, which is critical for sexual arousal. In a 14-day trial, men consuming double root coffee exhibited improved erectile function scores, linked to the synergistic effects of caffeine and tongkat ali on endothelial function and testosterone bioavailability (Zuma Coffee, 2025). They both have an impact on liver function. The African walnuts are hepatoprotective at moderate doses, and concerns about liver toxicity have been raised. A 2017 study on rats administered walnut leaf extract showed no significant alterations in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels, suggesting minimal hepatotoxicity (PMC, 2017). However, high-dose supplementation (≥ 400 mg/kg) caused mild hepatic inflammation in prolonged use, necessitating cautious dosing (Nwaichi et al., 2017). Double root coffee consumption is associated with improved liver health. A 2016 meta-analysis found that 2–3 cups of coffee daily reduced liver fibrosis risk by 30% and hepatocellular carcinoma incidence by 40% in individuals with chronic liver disease (PMC, 2016). The polyphenols in coffee, such as chlorogenic acid, mitigate oxidative liver damage and improve ALT/AST profiles (Advanced Urology Institute, 2025). However, excessive caffeine intake (>5 cups/day) may transiently elevate liver enzymes, underscoring the need for moderation (StatPearls, 2023).

5.1 CONCLUSION

African walnuts and double root coffee demonstrate promising roles in enhancing testosterone, libido, and sexual performance, with additional benefits for liver health when consumed in moderation. However, further human trials are needed to standardize dosing and evaluate long-term safety, particularly regarding hepatic impacts.

This study demonstrates that African walnut and double root coffee, whether administered singly or in combination, significantly enhance serum testosterone levels and exert hepatoprotective effects in Wistar rats. The results suggest that these natural products may have therapeutic potential in the management of male reproductive dysfunction and in maintaining liver health. However, higher combination doses appeared to yield diminishing hormonal benefits, highlighting the need for dose optimization.

Future research should focus on long-term safety assessments, mechanistic studies, and well-controlled human clinical trials to validate these findings and establish standardized dosing guidelines. In conclusion, African walnut and double root coffee present a promising natural approach to improving male reproductive health while safeguarding hepatic function.

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