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APHRODISIAC AND FERTILITY ENHANCING ACTIONS OF *'KUNU AYA'* (A BEVERAGE BLEND DEVELOPED FROM *CYPERUS ESCULENTUS, PHOENIX DACTYLIFERA AND COCOS NUCIFERA*) VIA TESTOSTERONE- BOOSTING, ANXIOLYSIS AND INHIBITION OF KEY ENZYMES ASSOCIATED WITH ERECTILE PROCESS

Idakwoji Precious Adejoh[1]*, Uzuazokaro Mark- Maria Agatemor[2], Nweje- Anyalowu Paul Chukwuemeka[3], Anyalogbu Ernest Anayochukwu Aniemeka[4] and Okafor Stephen Chiadika[5]

[1]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
[3]Department of Biochemistry, Clifford University, Owerrinta, Abia State, Nigeria.
[4]Department of Biotechnology, Federal University of Technology, Owerri, Imo State, Nigeria.
[5]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

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***Corresponding Author**
Idakwoji Precious Adejoh
Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

ABSTRACT

Sexual dysfunctions occur at a point in an adult's lifetime. To address this problem a number of therapeutic strategies have been developed, some of which are inadequate and/or plagued with side effects. Consequently, alternative methods which include the use of herbs with aphrodisiac and fertility- enhancing properties have been advocated. The present study investigated the mechanism of aphrodisiac and fertility- enhancing activities of aqueous extracts of *Cyperus esculentus, Phoenix dactylifera and Cocos nucifera* and *'Kunu aya'* (the beverage blend). The study was divided into *in vivo* and *in vitro* phases. In the *in vivo* phase, aphrodisiac and fertility- enhancing activities of the extracts were assessed in rats using physical/ behavioral (observation of mounting/ mating frequencies), biochemical (determination of sperm count, testosterone and PSA levels) psychological/ mood (anxiolytic effect using EPM models) methods while in the *in vitro* phase, different concentrations of each extract were subjected to phosphodiesterase- 5 (PDE-5), Arginase, Acetylcholinesterase

(AChE), Angiotensin Converting Enzyme (ACE) inhibition assays and their medium inhibitory concentrations (IC_{50}) determined through non-linear regression analysis. Results showed that the beverage blend significantly increased mounting/ mating frequency, sperm count, testosterone level, decreased PSA level and reduced anxiety. The blend was also more potent in inhibiting the enzymes compared to the individual extracts. It was then concluded that the possible mechanisms of aphrodisiac and fertility- enhancing properties of the individual extracts and their beverage blend- '*Kunu aya'* are via ability to increase testosterone level, increase sperm count, decrease anxiety and inhibition of key enzymes involved in erectile process.

KEYWORDS: Aphrodisiac, Anxiolysis, *Kunu aya, Cyperus esculentus, Phoenix dactylifera and Cocos nucifera.*

1.0 INTRODUCTION

Sexual activity is essential for procreation and the general wellbeing of humans. The inability to enjoy sexual intercourse is reffered to as 'Sexual dysfunction' and it may be categorized into sexual desire disorders, sexual arousal disorders, orgasmic disorders and sexual pain disorders.[1] A number of factors including psychological disturbances (performance anxiety, strained relationship, depression, stress, guilt and fear of sexual failure), deficiencies in sex hormones (testosterone deficiency),[2] chronic diseases (diabetes, hypertension, atherosclerosis, venous leakage),[3] neurological disorders (Parkinson's disease, Alzhemier's disease, spinal cord or nerve injury), side effects associated with chronic use of drugs (anti-hypertensives, central agents, psychiatric medications, antiulcer, antidepressants, anti-androgens), life style related complications (chronic alcohol abuse, cigarette smoking)[4] are usually implicated in sexual dysfunction.

Due to the 'embarrassing' nature of this condition, most patients are usually not outspoken about it to their physicians, hence, information about its prevalence is inconsistent. The prevalence of men sexual dysfunction is 56% in the United States of America while that of women is 63%. Prior to this development there were no data on female sexual dysfunction. Thirty-four (34%) of men between 40-70 years have one form of sexual dysfunction.[5] In a study to ascertain the prevalence of sexual dysfunction in male above 35 years in three countries, 80.8% of the study group in Pakistan had sexual dysfunction while, 57% of the men in Sub-Saharan Africa were reported to have the same sexual dysfunction.[6] In Nigeria, the prevalence of sexual dysfunction is 57.4% among men above 35 years.[7] Also reported

prevalence of sexual dysfunction of 38.5% and 63.9% among males between the ages of 31-40 and 61-70years respectively in South Western Nigeria.[8] Also reported that, sexual dysfunction has a prevalence of 58% among males with diabetes in Nigeria. Reproductive and sexual dysfunction is more pronounced with increase in age.[9] Erectile dysfunction is the most common form of sexual dysfunction that is rampant among sexually active men and it is estimated to affect about 322 million men globally by the year 2025.[10]

Erectile dysfunction is the consistent or recurring inability to attain or maintain a penile erection sufficient for vaginal penetration. Penile erection is a complex process that involves interplay of vascular, hormonal, psychological and neurological factors. Any condition leading to an alteration in any of these factors predisposes an individual to erectile dysfunction.[11] The inability to maintain a healthy sexual and reproductive life leads to depression, nervousness, anxiety, fear and ultimately low quality of life.

Aphrodisiacs and Fertility- enhancers are used in the management of sexual dysfunction. Aphrodisiacs are a group of substances that increase desire/ arousal, enhance/ improve penile erection or enhance sexual pleasure. Fertility- enhancers on the other hand promote fertility through their actions on sperm count, sperm morphology and motility, prostate health and hormonal balance in men for instance. Aphrodisiacs act in distinct mechanistic ways. For example, it has been suggested that cyclic-guanine monophosphate (cGMP), nitric oxide (NO) and acetylcholine (ACh) play active roles in the erection process.[12] [13] NO, cGMP and acetylcholine ACh are therefore targets for aphrodisiacs as low levels of these cellular messengers could lead to impaired erection.

The up-regulation of phosphodiesterase type 5 (PDE-5) reduces the levels of cGMP in penile tissue, thus impair penile erection.[14] In addition, the increased level of cGMP in penile tissues is dependent on NO-induced activation of guanyl cyclase; hence, maximum concentration of NO in the penile tissue is required.[15] In erectile dysfunction, the increased activity of arginase reduces production of NO, as arginase catalyzes the conversion of arginine to urea and ornithine, thereby reducing arginine levels that could be used for NO production by NO synthase (NOs). Arginase competes with NOs for L-arginine substrate, therefore, inhibition of arginase could up-regulate NO production.[16] [17]

Inhibition of Acetylcholinesterase (AChE) is another target for the treatment of erectile dysfunction as it regulates the levels of ACh, which trigger NO-dependent smooth muscle

relaxation in erection process.[18] The penile tissue is a reservoir of cholinergic nerves and ACh molecule.[19] However, the increase activity of AChE reduces the concentration of ACh by catalyzing the conversion of acetylcholine to acetate and choline, respectively.[20] As reported, inhibition of AChE increases the level of ACh, and consequently improves penile erection and rigidity.[21][22] Furthermore, the rennin–angiotensin system (RAS) is not only a major factor in the pathophysiology of hypertension but also in erectile dysfunction. The increase in the production of angiotensin-II as a result of elevated angiotensin-I converting enzyme (ACE) activity induces erectile dysfunction.[23 [24] [17] [20] Therefore, the inhibition of ACE activity in turn reduces angiotensin-II levels and improves erectile function in erectile dysfunction patients.[25]

The orthodox aphrodisiacs and fertility enhancers used for the treatment of sexual dysfunction include the Phosphodiesterase V inhibitors (Sildenafil, Tadalafil and Vardenafil), androgens (testosterone) and vasodilators (Aprostadil). The side effects associated with these synthetic drugs necessitated search for safer and effective aphrodisiac and fertility- enhancing agents especially of herbal origin. Medicinal plants represent an extraordinary reservoir of active ingredients. Aphrodisiac activities of medicinal plants from a number of medicinal systems have been reported. Plants with reported aphrodisiac properties include *Carica papaya, Cajanus cajan*,[26] *Cyperus esculentus*, *Phoenix dactylifera*[27] and *Cocos nucifera*.[28]

In some parts of Nigeria especially the North, a beverage blend referred to as '*Kunu aya'* is developed from the extracts of *Cyperus esculentus* (Tiger nut), *Phoenix dactylifera* (Dates) and *Cocos nucifera* (coconut) and taken for refreshment purposes. Apart from its refreshing properties, this beverage is also reputable for its aphrodisiac and fertility- enhancing actions. Though the individual components of this beverage have been proven scientifically to possess aphrodisiac and fertility enhancing properties, to the best of our knowledge, the actual mechanisms of action of these components have not been elucidated. Hence, the aim of this study was to examine the mechanism of aphrodisiac and fertility- enhancing actions of these components and their beverage blend using physical/ behavioral, biochemical and psychological/ mood methods and also using *in vitro* methods to investigate their modulatory effect on the activities of some key enzymes (phosphodiesterase V, arginase, acetyl cholinesterase and angiotensin converting enzyme (ACE)) involved in erectile process.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Drugs and Chemicals

Prostigmine, L- NOHA, Ethinyl estradiol, progesterone (Sigma Aldrich Chemical, USA). Sildenafil citrate and Lisinopril (purchased from Health Seal Pharmacy, Lokoja). Assay kit for prostate specific antigen (PSA), testosterone (DE-EIA ASSAY KIT) and corticosteroids (EIA ASSAY KIT) (purchased from Alpha Laboratories Ltd Abuja, Nigeria). All other chemicals used were of analytic grade.

2.1.2 Equipments

UV–visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom), Stop clock, elevated plus maze, animal cages.

2.2 Methods

2.2.1 Sample collection and preparation of extracts

Cyperus esculentus (Tiger nuts), *Phoenix dactylifera* (dates) and *Cocos nucifera* (coconut) were bought from International market', Lokoja, Kogi State, North Central, Nigeria and authenticated by an ethno- botanist at the Herbarium unit of the Department of Biological Sciences, Federal University, Lokoja. The samples were thoroughly washed, thereafter dried and pulverized, using electric blender. One thousand gram of each sample and their blend (Tiger nuts (600g) + dates (200g) + coconut (200g)) was weighed and extracted each with 2500 ml of distilled water. The filtrate was dried; thereafter each dried extract was stored in the refrigerator for subsequent *in vivo* and *in vitro* analyses. The extracts of *Cyperus esculentus*, *Phoenix dactylifera*, *Cocos nucifera* and their blend will henceforth be referred to as CE, PD, CN and KA respectively.

2.2.2 Experimental animals

One hundred adult Wistar rats (Female and male) weighing 180–250g were procured from the animal house facility of the Department of Biochemistry, Salem University, Lokoja and handled according to the guide for the Care and Use of Laboratory Animals, published by the National Institute of Health (NIH), USA. The rats were maintained at 25.0 ± 2^{O}C on a 12 h light/dark cycle with access to standard animal feed and water ad libitum for 7 d before the commencement of the experiment.

2.3 Acute Toxicity Study

The oral median lethal dose (LD_{50}) of the extracts was determined in rats according to the method of Lorke[29] with slight modifications using 18 rats for per extract. The study was carried out in two phases. In the first phase, 9 rats were divided into 3 groups of 3 rats each and were treated with the extract at doses of 10, 100 and 1000mg/kg body weight respectively after which they were observed for 24 hours for signs of toxicity and/ or mortality. Based on the results of the first phase, 9 rats were again divided into 3 groups of 3 rats each and were also treated with the extract at doses of 1600, 2900 and 5000 mg/kg body weight respectively in the second phase. The rats were also monitored 24 h after treatment and for signs of toxicity and/or mortality. The median lethal dose (LD_{50}) of each extract was estimated based on the observations in the second phase. This was done for each of the extracts.

2.4 *In vivo* Pharmacological Screening

2.4.1 Experimental design

Sixty (60) wistar rats randomized into ten (10) groups of six rats each were used for study. The rats were administered the drugs daily for 28 days as follows:

Group 1 (control group) received normal saline (10ml/kg)

Group 2 received 250 mg/kg CE

Group 3 received 500 mg/kg CE

Group 4 received 250 mg/kg PD

Group 5 received 500 mg/kg PD

Group 6 received 250 mg/kg CN

Group 7 received 500 mg/kg CN

Group 8 received 250 mg/kg KA

Group 9 received 500 mg/kg KA

Group 10 received Sildenafil citrate 5mg/kg b.wt

2.4.2 Physical method of assessing plant with aphrodisiac activity

2.4.2.1 Mounting frequency test

On the 24th day of the treatment each male rat in each group was put in a cage with two estrous female rats. Oestrous was induced in the female rats using 1mg progesterone and 100µg ethinylestradiol 6 and 48 hours respectively before the pairing.[30] The rats were

observed for mounting behavior. The number of times the male rat mounts the female within five minutes time frame was counted and recorded

2.4.2.2 Mating frequency test

On the 26th day of treatment the sexual episode/intromission is usually established when a male rat mount a female rat and lick its penis. The number of times each male rat in all the groups mounted a female and licked its penis was recorded for a period of five minutes.[30]

2.4.3 Biochemical method of assessing plants with aphrodisiac and fertility effects.

2.4.3.1 Collection and separation of sera samples for biochemical analyses.

On the last (29th) day of the experiment, overnight fasted rats were anaesthetized by diethyl ether inhalation. Blood samples were collected via cardiac puncture into plain sera tubes and allowed to clot. Serum was separated by centrifugation using Denley BS400 centrifuge (England) at 3000 rpm for 10 minutes and then assayed for levels of biochemical parameters. The testes and seminal vesicles were thereafter dissected and an incision was made at the caudal epididymis from where semen samples were collected for analyses.

2.4.3.2 Sperm count

The caudal epididymidis was cut open and washed with physiological saline; sperm were collected by diffusion of sperm. The epididymidis was put in petri-dishes containing physiological saline preheated to 37^0C and the epididymis were extruded gently and fluid flow into the petri-dishes which is then incubated for 10 min in 37^oC in a constant temperature incubator. Each semen sample collected was evaluated microscopically for sperm count.

2.4.3.3 Corticosteroid assay

The corticosteroid assay was carried out according the method[31] using EIA assay kit.

2.4.3.4 Prostate specific antigen (PSA) assay

The prostate specific antigen (PSA) assay was also carried out using the method described by.[32] DIASPOT PSA assay kit, a prostate specific antigen semi-quantitative rapid test trips was used.

2.4.3.5 Testosterone assay

The method described by[31] in which the serum is used to ascertain the testosterone level. The DS-EIA STEROID-TESTOSERONE-RT immunoenzymo-metric assay kit was used.

2.4.4 Psychological method of assessing plants with aphrodisiac and fertility effect

2.4.4.1 *Anxiety study*

The anxiety test was carried out based on a method by.[33] On the 27th day the rats were subjected to anxiety test and were observed for 5 minutes to ascertain how much 40 time was spent in the open and closed arm. Six rats from each group were subjected to the EPM by placing it at the center of the maze and observed for five minutes; the EPM was swapped clean with ethanol and another rat was observed. The open and close arm entries were recorded; percentage entries into the closed arm and percentage into the open arm and the time spent were recorded and were used to measure anxiety indices. The close arm entries were used to deduce locomotors activities.

2.4.4.2 *Learning and memory studies*

This was based on a modified method described by.[34] This test measures the effect of the extracts on memory and cognition. The rats were subjected to the elevated plus maze test (EPM), on the 7th, 14th and 21st day for 90 seconds; the time spent by the rats in the open arm was recorded.

2.5 *In vitro* Pharmacological Screening

2.5.1 Inhibition of phosphodiesterase-5 (PDE-5) activity assay

The penile tissue was carefully removed and homogenized with three volumes of ice-cold buffer [0.1M Tris-HCl buffer containing 1mM $CaCl_2$ and 50mM NaCl (pH 8.0)]. The ability of the extracts to inhibit PDE-5 activity was assessed.[35] The reaction mixture containing 5mM of the substrate (p-nitrophenyl phenylphosphonate), tissue homogenate, 20mM Tris buffer (pH 8.0) and the extracts/sildenafil were incubated at 37^{o}C for 10 min. The intensity of p-nitrophenol produced was measured as a change in absorbance after 5 min at 400 nm. The control experiment was performed without the extracts/sildenafil. The PDE-5 inhibitory activity was expressed as percentage inhibition using the formula below:

PDE- 5 inhibition (%) = $[(Abs_{control} - Abs_{samples})/ Abs_{control} \times 100$ (a)

Where Abs control is the absorbance without the extract and Abs samples is the absorbance with extract.

2.5.2 Inhibition of arginase activity assay

Penile tissue was homogenized with three volumes cold buffer (phosphate buffer, pH 7.2), and centrifuged for 20 min at 357.80g. The supernatant was used as a source of arginase, in

which the activity was determined using[36] method in a reaction mixture containing Tris–HCl buffer (1.0 mM, pH 9.5, 1.0mM MnCl), 0.1M arginine solution and extract/L-2-amino-[4-(20-hydroxyguanidino)] butyric acid (L- NOHA). The mixture was made to a final volume of 1.0 ml. The mixture was incubated for 10 min at 37^{o}C. The reaction was terminated by the addition of 2.5ml Ehrlich reagent [2.0 g of p- dimethylaminobenzaldehyde in 20mL of absolute hydrochloric acid (37% purity) and made up to 100mL with distilled water]. The absorbance was read after 20 min at 450 nm. The control experiment was performed without the test sample or standard and arginase inhibitory activity was calculated and expressed as % inhibition [Equation (a)].

2.5.3 AChE inhibitory assay

Homogenate of the rat penile tissue was prepared in three volumes of cold buffer (phosphate buffer, 0.1 M, pH 7.2) and used as the source of AChE (EC 3.1.1.7). The effect of the extracts/ prostigmine on AChE activity was assessed using colorimetric method.[20] The AChE activity was determined in a reaction containing of 200 µL tissue homogenate, 100 µL of 5,50-dithio-bis(2-nitrobenzoic) acid (DTNB 3.3 mM), extracts or prostigmine and phosphate buffer, pH 8.0. The mixture was incubated for 20 min at 25^{o}C and the substrate (acetylthiocholine iodide) was added. Immediately, the enzyme activity was measured at 412 nm. The AChE activity was thereafter expressed as % inhibition using Equation (a).

2.5.4 Angiotensin converting enzyme (ACE) inhibitory assay

The effect of the extracts on ACE activity using the method of[20] was investigated. The extracts/ Lisinopril and 50 µL of the penile homogenate as a source for ACE (EC 3.4.15.1) were preincubated at 37^{o}C for 15 min. The ACE substrate [150 µL, 8.33mM hippuryl-l-histidyl- leucine in 125mM of Tris– HCl buffer (pH 8.3)] was added to the mixture which was incubated at 37^{0}C for 30 min. The reaction was halted with 250 µL of 1M HCl. The enzymatic product [hippuric acid (Bz- Gly)] was extracted with 1.5 ml of ethyl acetate and centrifuged to separate the ethyl acetate layer. Thereafter, 1ml of ethyl acetate layer was transferred to a clean test tube and evaporated to dryness. Distilled water (1 ml) was added and its absorbance was measured at 228 nm. The control experiment was performed without the test sample/ Lisinopril. The percentage ACE inhibition was subsequently calculated (Equation (a)).

2.6 Data analysis

All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by Newman Keuls post-hoc. P ≤0.05, ≤ 0.01 and ≤0.001 were considered significant. The extract concentration causing 50% enzyme activities (IC_{50}) value was determined using non-linear regression analysis with Graph Pad Prism version 5.00 (Graph Pad Inc.,).

3.0 RESULTS

3.1 Acute Toxicity Studies

The LD_{50} was taken to be ≥ 5,000mg/kg for all the extracts since no mortality was recorded at doses up to 5000 mg/kg. No toxic sign was also observed during the acute toxicity test.

3.2 Physical Assessment of Extracts for Aphrodisiac Properties

3.2.1 Mounting frequency test

There was a significant (P≤0.05) increase in mounting frequency in the CE- treated groups compared to the control group. At 250 mg/kg KA, there was significant (P≤0.05) increase in mounting frequency compared to the control. The most significant (P≤0.001) increase in mounting frequency was observed in the group that received 500mg/kg of KA (Figure1).

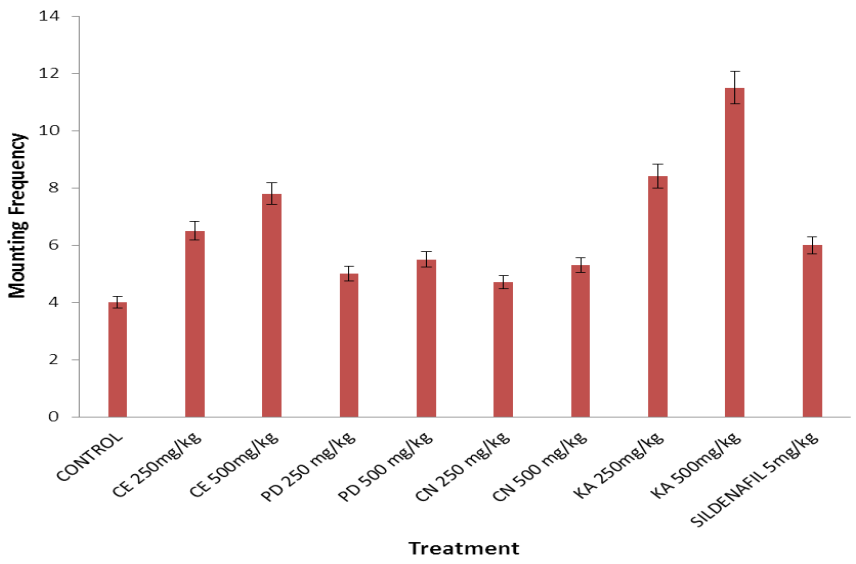

Figure 1: Effect of CE, PD, CN and KA on male mounting frequency in male rats. *P≤ 0.05, **P≤0.01, ***P≤0.001 compared to the control. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.

N = 6, SEM: Standard Error of Mean, ANOVA and Newman- Keuls Post hoc test

3.2.2 Mating frequency test (sexual episode)

There was significant (P≤0.05) increase in mating frequency in rats treated with 250mg/kg and 500 mg/kg CE compared to the control. KA produced significant (P≤0.01) and (P≤0.001) at doses of 250mg/kg and 500 mg/kg respectively compared to the untreated control which was comparable to the effect of Sildenafil (Figure 2).

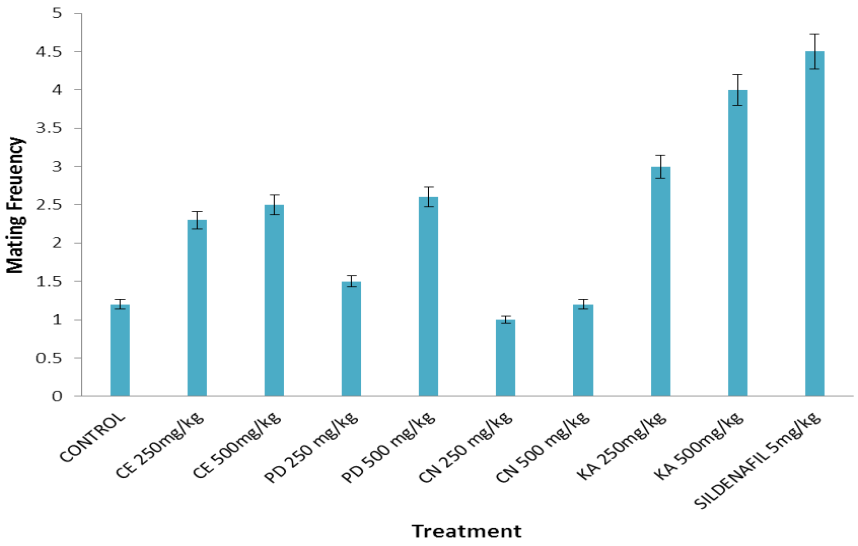

Figure 2: Effect of CE, PD, CN and KA on male mating frequency in male rats. *P≤ 0.05, **P≤0.01, ***P≤0.001 compared to the control. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.

N = 6, SEM: Standard Error of Mean, ANOVA and Newman- Keuls Post hoc test

3.3 Effect of Extracts on Biochemical Parameters

3.3.1 Effect of extracts on sperm count

Only KA at 500mg/ kg produced a significant (P≤0.05) increase in sperm count in the treated rats compared to the control (Figure 3).

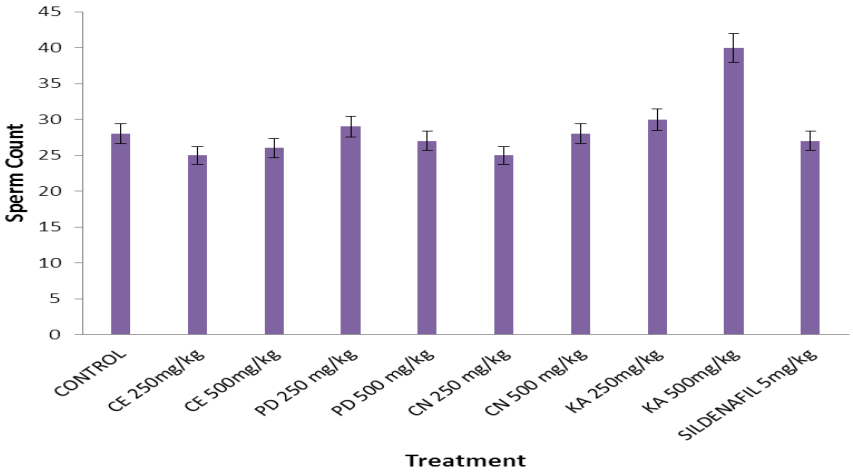

Figure 3: Effect of CE, PD, CN and KA on sperm count in male rats. *P≤ 0.05, **P≤0.01, ***P≤0.001 compared to the control. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.
N = 6, SEM: Standard Error of Mean, ANOVA and Newman- Keuls Post hoc test

3.3.2 Effect of the extracts on testosterone level

CE at doses of 250 and 500 mg/ kg produced significant (P≤0.01) increase in serum level of testosterone compared to the control. PD also at the same doses produced significant (P≤0.05) increase in serum level of testosterone compared to the control. KA however, at doses of 250 and 500 mg/ kg produced significant (P≤0.001) increases in serum level of testosterone that were comparable to the effect of sildenafil citrate (Figure: 4).

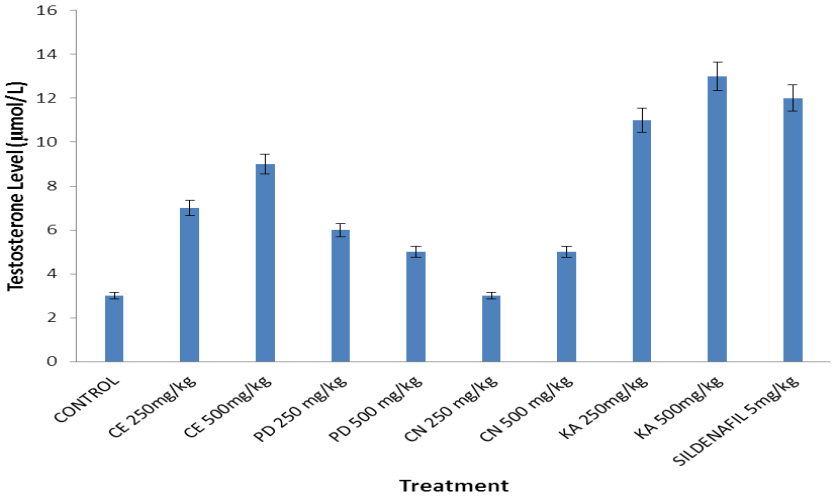

Figure 4: Effect of CE, PD, CN and KA on serum testosterone level in male rats. *P≤ 0.05, **P≤0.01, ***P≤0.001 compared to the control. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.
N = 6, SEM: Standard Error of Mean, ANOVA and Newman- Keuls Post hoc test

3.3.3. Effect of the extracts on cortisol level

There was a dose dependent decrease in cortisol level in all the treated groups though not statistically significant. However, there was a significant (P≤0.05) increase in cortisol level in sildenafil- treated rats compared to the control group (Figure: 5).

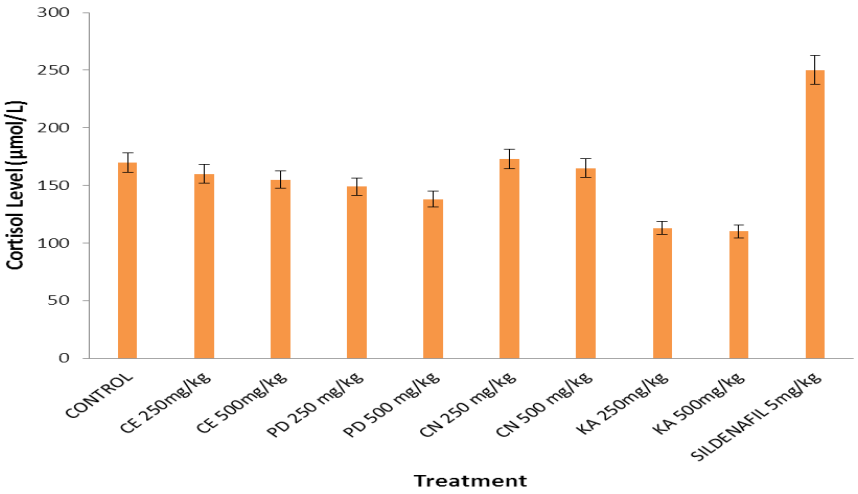

Figure 5: Effect of CE, PD, CN and KA on serum cortisol level in male rats. *P≤ 0.05, **P≤0.01, ***P≤0.001 compared to the control. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.
N = 6, SEM: Standard Error of Mean, ANOVA and Newman- Keuls Post hoc test

3.3.4 Effect of the extracts on prostate specific antigen (PSA) level

The extracts apart did not produce significant effect on the PSA levels. (Fig: 6).

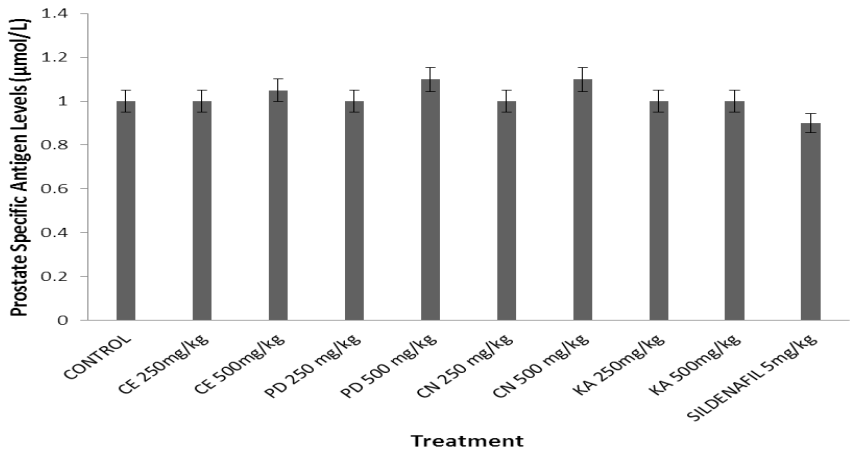

Figure 6: Effect of CE, PD, CN and KA on serum Prostate Specific Antigen (PSA) level in male rats. *P≤ 0.05, **P≤0.01, ***P≤0.001 compared to the control. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.
N = 6, SEM: Standard Error of Mean, ANOVA and Newman- Keuls Post hoc test

3.4 Psychological Effects of the Extracts

3.4.1 Effect of the extract on anxiety

The CE, PD and KA treated rats showed significant (P≤0.01) increase in time spent in the open arm at 250mg/kg and 500mg/kg doses. There was corresponding significant decrease (P≤0.001) in time spent in the closed arm of the maze in rats treated with these same extracts at the same dose levels compared to the control rats. Similarly, the sildenafil citrate treated rats showed significant (P≤0.001) increase in time spent in the open arm. CN at doses of 250 and 500mg/kg showed no significant effect in the time spent in both the open and closed arms of the maze (Figure 7).

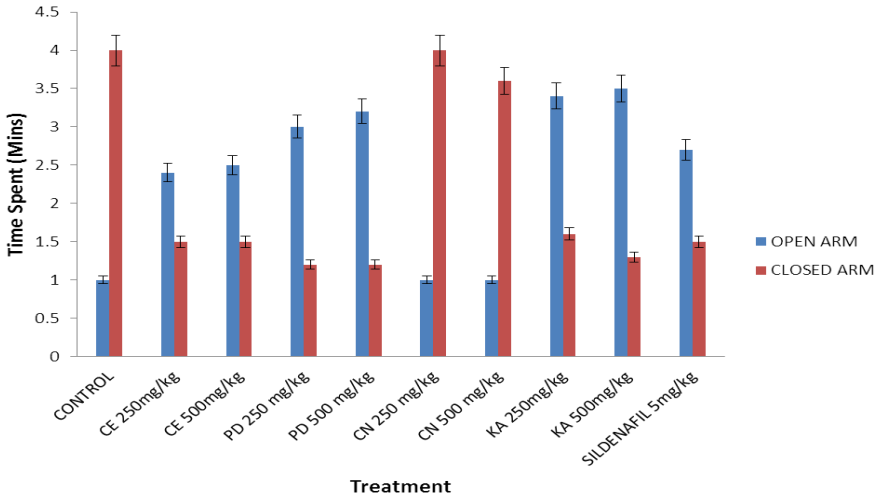

Figure 7: Effect of CE, PD, CN and KA on the time spent in the open and closed arm of the elevated plus maze. *P≤ 0.05, **P≤0.01, ***P≤0.001 compared to the control. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.

N = 6, SEM: Standard Error of Mean, ANOVA and Newman- Keuls Post hoc test

3.4.2 Effect of the extracts on cognition

All the extracts at 250 and 500 mg/kg and sildenafil citrate at 5mg/ kg significantly (P≤0.001) increase the time spent on the open arm by rats from the 7th day to the 14th day compared to the control (Figure 8).

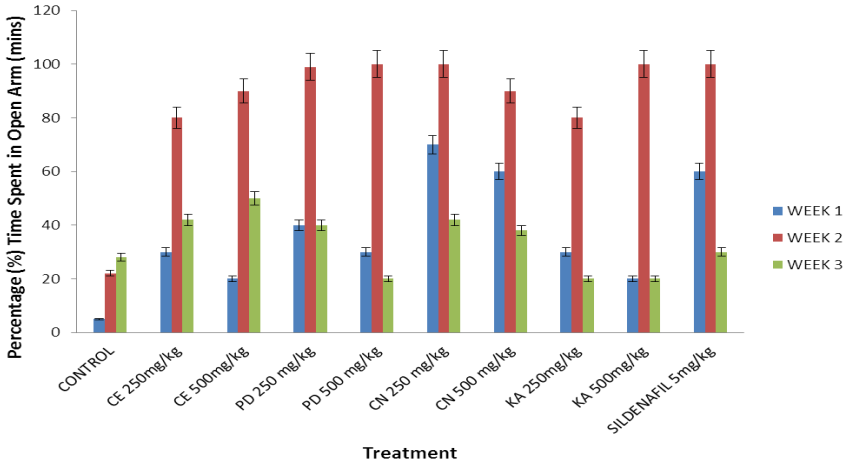

Figure 8: Effect of CE, PD, CN and KA on memory/cognition in rats. *P≤ 0.05, **P≤0.01, ***P≤0.001 compared to the control. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.

N = 6, SEM: Standard Error of Mean, ANOVA and Newman- Keuls Post hoc test

3.5 Inhibitory Effect of Various Concentrations of the Extracts on the Activity of Phosphodiesterase- 5 (PDE- 5)

The extracts produced concentration- dependent inhibitory effect on PDE- 5 activity. The median inhibitory concentration (IC_{50}) of CE, PD and CN were estimated to be 106.7 µg/ ml, 61.95 µg/ ml, 223.0 µg/ ml respectively while KA had a value of 34.45µg/ ml which was the closest to sildenafil citrate (96 µg/ ml).

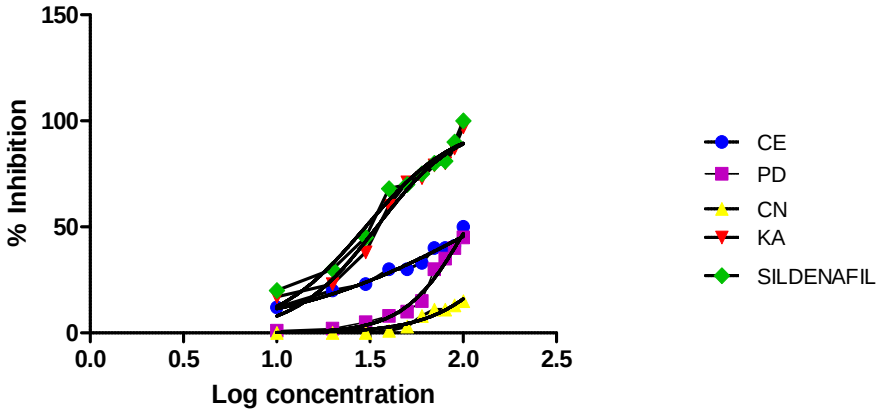

Figure 9: Concentration- Percentage Inhibition Curve for CE, PD, CN and KA for PDE V. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.

3.6 Inhibitory Effect of Various Concentrations of the Extracts on the Activity of Arginase

The extracts also produced concentration- dependent inhibitory effect on arginase activity. The median inhibitory concentration (IC_{50}) of CE, PD, CN and KA were estimated to be 74.01 µg/ ml, 92.20 µg/ ml, 149.5 µg/ ml and 61.80µg/ ml respectively while the standard L-NOHA had an IC_{50} of 29.96 µg/ ml.

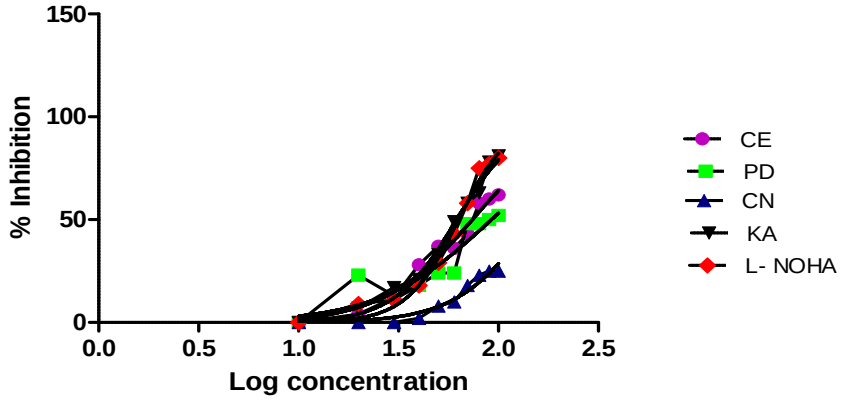

Figure 10: Concentration- Percentage Inhibition Curve for CE, PD, CN and KA for Arginase. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.

3.7 Inhibitory Effect of Various Concentrations of the Extracts on the Activity of Acetylcholinesterase

A concentration- dependent inhibitory effect on acetylcholinesterase activity was observed. CE, PD and CN had median inhibitory concentration (IC_{50}) of 62.23 µg/ ml, 94.10 µg/ ml and 145.3 µg/ ml respectively while KA had a value of 38.52µg/ ml which was the closest to prostigmine (28.15 µg/ ml).

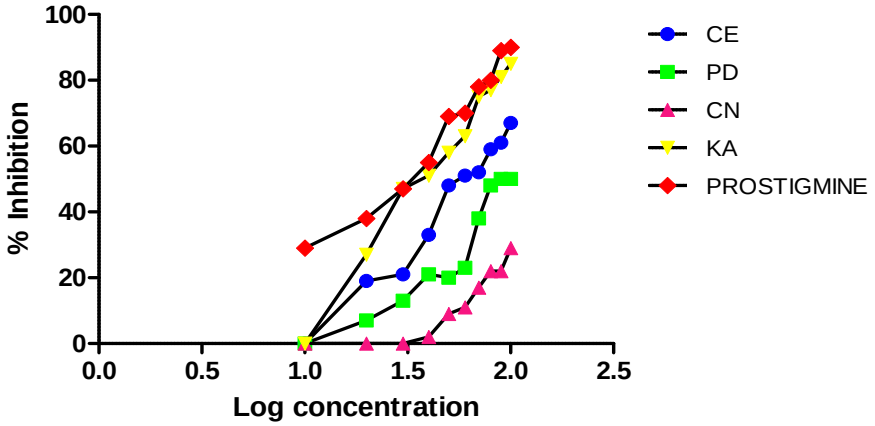

Figure 11: Concentration- Percentage Inhibition Curve for CE, PD, CN and KA for Acetylcholinesterase. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.

3.8 Inhibitory Effect of Various Concentrations of the Extracts on the Activity of Angiotensin Converting Enzyme (ACE)

A concentration- dependent inhibitory effect on ACE activity was observed. The median inhibitory concentration (IC_{50}) of CE, PD, CN and KA were estimated to be 70.34 µg/ ml, 117.5 µg/ ml, 113.5 µg/ ml and 41.78µg/ ml respectively while the standard Lisinopril had an IC_{50} of 27.65 µg/ ml.

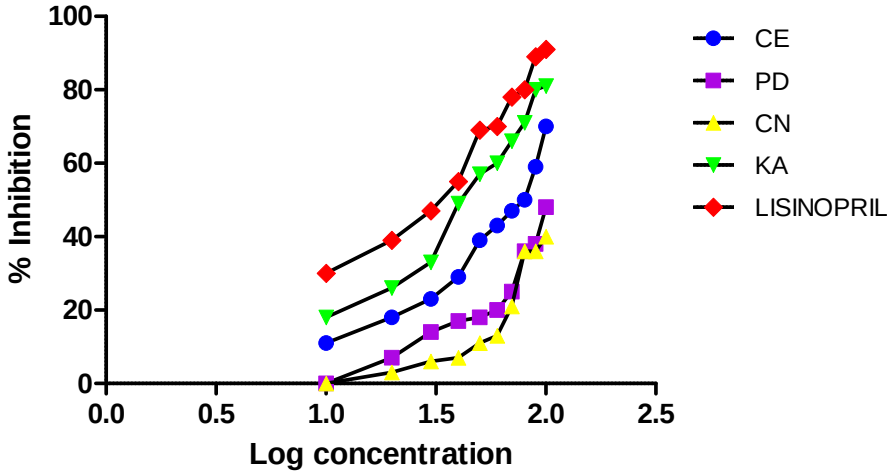

Figure 12: Log Concentration- Percentage Inhibition Curve for CE, PD, CN and KA for ACE. CE= *Cyperus* esculentus, PD= *Phoenix dactylifera*, CN= *Cocos nucifera*, KA= Kunu aya (a beverage blend made from CE, PD and CN) aqueous extracts.

4.0 DISCUSSION

Sexual dysfunctions (decreased libido, erectile and ejaculatory dysfunction) occur at a point in an adult's lifetime due to factors such as psychological disorders (anxiety, depression, stress and fear of sex), neurological disorders (stroke, cerebral trauma, Alzheimer and Parkinson's disease), chronic disorders (diabetes, hypertension, vascular insufficiency and atherosclerosis), penile disease (phinosis, peyronies, ballanitis and priapism), life style (chronic alcohol abuse and cigarette smoking) and decrease in hormone level with age. Information on the use of aphrodisiac dates back to millennia because these conditions are almost as old as man. Aphrodisiacs can be any kind of food, drug, scent or device that can arouse or increase sexual drive or libido. They can therefore, be described as substances that enhance sexual drive or libido.[37]

This study investigated the aphrodisiac and fertility- enhancing actions of a beverage blend referred to as '*Kunu aya'* which is consumed for refreshment purposes in some parts of Nigeria especially the North. This beverage is developed from *Cyperus esculentus* (Tiger nut), *Phoenix dactylifera* (Dates) and *Cocos nucifera* (Coconut). Apart from its refreshing properties, it is also reputable for its aphrodisiac and fertility- enhancing actions. Though the individual components of this beverage have been proven scientifically to possess aphrodisiac and fertility enhancing properties, there is dearth of information as regards their actual mechanisms of action. This study examined the modulatory effect of the aqueous extracts of *Cyperus esculentus*, *phoenix dactylifera*, *Cocos nucifera* and their blend on some physical, biochemical and psychological aspects of sexuality and also established their inhibitory action on the activities of some key enzymes (phosphodiesterase V, arginase, acetylcholinesterase and angiotensin converting enzyme (ACE)) implicated in erectile dysfunction.

The physical (behavioral) methods employed in this study were mount and mating (intromission) frequencies. In males, increased mounting frequency is considered as an indication of sexual arousal and desire.[38] Disorder of sexual desire (libido) can involve either a deficient or compulsive desire for sexual activity and may include hypoactive sexual desire (HSD), a persistent or recurrent deficient or absence of sexual fantasy and desire for sexual activity.[39] The significant increase in mounting frequency observed with the extract-treated rats compared to the control group indicates an increase in sexual desire thus aphrodisiac activity. There was also an increase in mating frequency as observed across the

groups. The significant increase in mating frequency produced by the extracts implies aphrodisiac properties specifically, arousal, motivation and vigor which enable penetration and consequently sexual intercourse.[40] The increase in mating frequency was more pronounced in the group treated with the extract of the blend at a dose of 500mg/ kg, the effect at this dose is comparable to that of sildenafil, the standard drug used in the studies. Fertility abnormalities are associated with low sperm count and abnormal sperm cells.[41] Fertilization therefore, requires adequate and normal sperm count, morphology and motility to occur. The observed significant increase in the blend extract-treated rats compared to the control group indicates that the extract has fertility - enhancing effect.[40] Reported that male sexual dysfunction is associated with testosterone deficiencies. Testosterone supplementation (hormone replacement therapy) has been shown to improve sexual function, libido, intensity of orgasm and ejaculation. A plant extract capable of increasing the level of testosterone is thus considered an aphrodisiac. The level of testosterone increased across the groups treated with the extracts but more significant in the blend- treated groups compared to the control indicating aphrodisiac properties. This may be a probable mechanism of aphrodisiac activity of the extract.

Cortisol also known as ‘stress hormone’ is released from the adrenal cortex in response to oxidative stress.[42] Feeling of stress may be accompanied by suppressed libido, production of gametes and reduction in the frequency of sexual intercourse, fertilization, implantation and maintenance of pregnancy.[43] High level of cortisol has negative effects on sexual function by lowering sex drive (libido) and nocturnal penile erection. Stress may also have a negative influence on semen quality.[44] [45] The observed decrease in cortisol levels in the extract-treated rats compared to the control group is an indication of fertility- enhancing ability.

Prostate specific antigen (PSA) is a biomarker of the integrity of the prostate. It is secreted into the semen to reduce its viscosity thus facilitating spermatozoa escape and ensuring fertilization.[46] Hyperviscosity of semen has been associated with ejaculatory disorders (painful ejaculation, severe retarded or absent ejaculation) and low sperm count.[47] Normal semen rarely prevents sperm movement; however, hyper- viscous semen produces impaired trapping effect due to the visco-elasticity of the seminal plasma that inhibits normal sperm motility.[48] Very little PSA escapes from a healthy prostate into the blood thus increased PSA in blood is associated with erectile dysfunction. All the extracts apart from the blend had no

significant effect on PSA level compared to the control indicating that the extracts do not adversely affect semen viscosity in the rats.

The psychological toll of sexual dysfunction has been associated with anxiety which is excessive worry about everyday life activities which may result in distress and significant impairment of normal activity. Neurobiological expression of anxiety, though complex, mainly involves a release of adrenergic substances (adrenaline and noradrenaline). Increased release of adrenergic substance in turn affects arousal and orgasmic phases negatively and this may interfere with sexual arousal desire. According to[49] drugs that increase open arm exploration on the EPM have anxiolytic effects. Since there was an observed increase in the amount of time spent by the rats in the open arm of the EPM, It is safe to say that the extracts possess anxiolytic effect and by extension beneficial in sexual dysfunction with anxiety as the underlying cause.

Phosphodiesterase-5 (PDE-5), Arginase, Acetylcholinesterase (AchE) and Angiotensin Converting Enzyme (ACE) are important enzymes in the erection process. PDE-5 catalyzes the breakdown of the cGMP and also reduces NO levels in the endothelial cells, thereby decreasing signaling.[14] PDE-5 inhibitors such as sildenafil do not only raise the levels of cGMP[50] [51] but also stimulates activation of cGMP and increases NO bioavailability for the relaxation of penile tissue that results in penile erection. However, these inhibitors also elicit several side effects, including headache, dyspepsia, nasal congestion, visual abnormalities among others.[52] In this study, it was observed that PDE-5 activity was inhibited by the extracts individually and as a blend. The extract of *Cocos nucifera* showed the lowest potency against PDE- 5 as observed in its IC_{50} value (223.0 µg/ ml) while the blend was the most potent among the extracts (IC_{50} value of 34.45µg/ ml) which comes closest to that of the standard Sildenafil (IC_{50} value of 29.96 µg/ ml).

This finding is in line with previous reports that medicinal plant extracts can inhibit PDE-5 activity, especially plants rich in flavonoids.[53] [54] The utilization of L-arginine by arginase activity in vascular endothelia and smooth muscle cells of penile tissue can reduce NOS activity and consequently decrease concentration of NO: a major factor in erectile function.[55] Hence, the inhibition of arginase activity can be beneficial in the management of erectile dysfunction as this could increase the bioavailability of L-arginine, contribute to the production of NO via reaction catalyzed by NOS, where overall result could facilitate penile erection. In this study, arginase activity was inhibited by the extracts and their blend. The

extract of *Cocos nucifera* has the lowest potency as evident in its IC_{50} value of 149.5 µg/ ml while the blend has the highest potency with IC_{50} value of 61.80 µg/ ml which is comparable to that of the standard L- NOHA (IC_{50} value of 63.03 µg/ ml). This observed inhibitory effect of the plant extracts might be as a result of the presence of phenolics including epicatechin, quercetin, quercitrin and isoquercitrin. As it has been reported before that plant extracts rich in these phytochemicals exhibited arginase inhibitory potential.[56] [17] [20]

Penile tissue is rich in cholinergic nerves which release ACh for the stimulation of NO production from L-arginine by the catalytic action of neuronal nitric oxide synthase (nNOS) for sexual stimulation to occur.[18] The inhibition of AChE by the extracts correlates with recent studies that plant derived AChE inhibitors can be employed in ameliorating erectile dysfunction and can also prevent oxidative stress in neuronal cells of penile.[21] [22] [20] The inhibition of AChE by the extracts could be associated with the presence of phenolic compounds. This agrees with recent studies that phenolic-rich plants could inhibit AChE.[20] [57] The extract of *Cocos nucifera* showed the lowest ability to inhibit AchE as evident in its IC_{50} value of 145.3 µg/ ml while the blend also showed the highest potency with IC_{50} value of 38.52 µg/ ml which comes closest to that of the standard prostigmine (IC_{50} value of 28.15 µg/ ml).

Angiotensin-II, apart from its role in increase in hypertension, is also implicated in erectile dysfunction.[58] [20] Therefore, any agent that prevents the production of angiotensin- II could be useful in the management of hypertension and hypertensive induced erectile dysfunction. ACE is the enzyme responsible for the production of angiotensin- II and its inhibition does not only stop the production of angiotensin- II but also activate the release of NO and bradykinin; an active biomolecule in erectile function process.[17] [20] The inhibition of ACE activity in this study could be a function of interaction between the phenolics in the extracts with disulphide bridge of the enzyme and/or chelation of zinc atom within the active site of the enzyme.[58] [59] This is in line with the studies of[17] [59] [20] that plant extracts rich in phenolic compounds inhibited ACE activity. The extract of *Phoenix dactylifera* showed the lowest potency in the inhibition of AchE as it was observed to have the highest IC_{50} value (117.5 µg/ ml) while the blend was the most potent among the extracts (IC_{50} value of 38.52 µg/ ml) which comes closest to that of the standard Lisinopril (IC_{50} value of 27.65 µg/ ml).

5.0 CONCLUSION

It can be concluded from the results of this study that the possible mechanisms of aphrodisiac and fertility- enhancing properties of *Cyperus esculentus*, *Phoenix dactylifera*, *Cocos nucifera* extracts and their beverage blend- '*Kunu aya'* include the ability to increase testosterone level and sperm count, reduce stress and anxiety and also inhibit key enzymes involved in erectile process. Nevertheless, the beverage blend appeared to be the most potent possibly through additive interaction and may play an important role as an alternative remedy in the treatment of sexual dysfunction.

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