

SEXUAL INVIGORATING POTENTIALS OF COMBINED CRUDE EXTRACTS OF *SABICEA CALYCINA* AND *CARPOLOBIA LUTEA* ON MALE WISTAR RATS IN CRUDE OIL CHALLENGED ENVIRONMENT.

Abstract

The effects of 50, 100 and 200 mg/kg of combined ethanol extract of *S. calycina* and *C. lutea* was investigated on mating behavioural parameters and sex/reproductive hormone boosting potentials in both non-bonny light crude oil (NBLCO) and bonny light crude oil (BLCO) environmentally challenged rats. Forty sexually experienced rats weighing 120-135g were arbitrarily allocated into two groups of twenty rats as NBLCO and BLCO respectively, each group were further subdivided into four groups of five rats and administered orally with 50 mL distilled water (control) and the extract of *S. calycina* and *C. lutea* at doses of 50, 100 and 200 mg/kg body weight. After 21 days of treatment protocols, the animals were observed for both precopulatory and copulatory activities, sex hormone was also evaluated. The copulatory activities monitored showed that mount latency, intromission latency and post ejaculatory intervals were significantly decreased by the 100 and 200 mg/kg of the extract in both the NBLCO and BLCO challenged rats ($P < 0.05$). All doses significantly increased ($P < 0.05$) the mount frequency, intromission frequency and ejaculatory frequency in the NBLCO groups while only the 100 and 200 mg/kg body weight was able. Copulatory efficiency was significantly increased ($P < 0.05$) by all doses in a dose related manner in the NBLCO groups while only the 200 mg/kg dose level was able to significantly increase ($P < 0.05$) the copulatory efficiency in the BLCO groups. Hormonal assay showed that all doses produced significant increase ($P < 0.05$) in testosterone, luteinizing hormone, follicle stimulating hormone and progesterone, with a concomitantly significant decrease ($P < 0.05$) in prolactin in the NBLCO groups, however, only the 100 and 200 mg/kg was able to achieve significant change ($P < 0.05$) in these hormonal levels in the BLCO groups. Findings from this study showed that the extract has aphrodisiac potentials and also the ability to restore hyposexual activity in rats challenged with crude oil.

Keywords: Aphrodisiac, *C. lutea*, *S. calycina*, copulatory activity, sex hormones.

1.0 Introduction

The South–South Geocological Zone also known as the Niger Delta of Nigeria is known for its vast reserves of oil and gas deposits coupled with its opulent vegetation and wildlife. Abubakaret *al.* [1] opined that the region is indisputably responsible for the revenue that accrues to the Nation as petroleum account for over 80% of the government revenue, provides more than 95% of export incomes as well as generates over 40% of the country's Gross Domestic Product (GDP). With much of this oil and gas reserves still yet untapped, the region produces 2.2 million barrels of oil per day [2] of which an average of 240,000 barrels are spilled into the zone [3]. It is also projected that an average of 11-54 mg/L of the oil is dissolved in coastal waters in the region [4]. The spills and flares of gas results in pollution of surface and ground water, atmospheric air and crops with hydrocarbons including recognised carcinogens like polycyclic aromatic hydrocarbons (PAH) e.g benzo (a) pyrene, naturally occurring radioactive materials (NORM) e.g. uranium and thorium; and trace metals that are further bioaccumulated in some food crops. The individual and synergistic effects of this pollutants often

herald both acute and chronic health concerns in the region which includes haemotoxicity [5,6], hepatotoxicity [7], carcinogenesis through its effects on chromatin DNA [8], reduced fertility and sexual dysfunction [9,10,11] and systemic diseases which may in turn affect male sexual function [12].

Sex is one characteristic of human life that has continuously held a position of prominence at every time and within every culture nearly without exception, it is not just for procreation and ease of sexual tension on the part of the man but above all, be capable of quenching and satisfying the sexual urge of his partner. With the increasing incidence of male sexual dysfunction globally most often characterised by erectile dysfunction and premature ejaculation, it is reported that nearly 100 million persons around the world are living with it [13,14]. It is also estimated as having a prevalent rate of 10% across all ages [15], the environmental toxicants in the zone arising crude oil exploration and spills may even contribute toward an increased prevalent rate in the South–South geocological zone [16,17]. In Nigeria, the National Health Policy focuses on services like malaria, family planning, diseases like STI/HIV/AIDS, infant and maternal health exclusive of men's sexual health, although it may not be life threatening, the fact that the man cannot fulfill his conjugal responsibility can take a heavy psychological toll, causing depression, anxiety, debilitating feeling of inadequacy and low self-worth and may thus further contribute in heightening the tension in the region. Women whose spouse suffer sexual dysfunction may be tempted to go outside their marriage vows to satisfy their sexual cravings and needs and this can lead to increase HIV/AIDS exposure, broken homes and marriages with an overall adverse consequence on the society. Sexuality, a key determinant of the quality of life is an vital element of emotional and physical intimacy that most people crave to experience throughout their life time, unfortunately this may sometimes be a mirage to many, most especially the male folk due to male sexual dysfunction. Since ancient times human societies have searched for drugs and methods to induce sexual arousal, heighten their sexual experience and improve their experience. Throughout history many preparations from plants and animals have been reputed to have these properties e.g. Yohimbine, mandrake plant, ginseng, ground Rhinoceros horn, sheep/bull testicles, Spanish fly, *Zanthoxylum lepreuri* etc.[18,19]. The emergent discipline of sex research has however spawned a more specific field called Pharmacosexology which is the study of the effects (both positive and negative) of various ingredients on sexual arousal. These substances that affect sexual arousal and performance are collectively called Aphrodisiacs[20]. Aphrodisiacs are of two classes namely psychophysiological stimuli which are typically photographic, perceptible, olfactory and aural preparations, the other which are internal preparations include food, alcoholic drinks, drugs and love potions [21]. Sex is a complex, multi-dimensional phenomenon that incorporates biological, psychological, interpersonal and behavioural dimensions [22]. Male sexual stimulation results in sequences of psychosomatic, neuronal, vascular and local genital modifications. Not less than three

different classifications for these changes have been depicted. Kolodny *et al.* [23]. described a psychosexual response cycle that consist of four phases namely excitement, plateau, orgasm and resolution. In another classification the psychosexual phase is divided into two interconnected activities as excitement into latency and tumescence, plateau into erection and rigidity, orgasm into emission and ejaculation and resolution into detumescence and refractoriness [24]. The third classification focuses on the functional activities during sexual cycle [25]. It adds an initial phase of desire and libido to encompass the sex seeking behaviour, pools together excitement and plateau into a single phase of erection and splits the orgasmic phase into the physical function of ejaculation and the psychological sensation of orgasmic pleasure. Thus, the normal male sexual response cycle can be effectively divided into five interconnected events that occur in a defined sequence namely Libido, erection, ejaculation, orgasm and detumescence. Sex disorders of the male are classified into disorders of sexual function, sexual orientation and sexual behaviour. Disorder of sexual orientation and behaviour are believed to be entirely due to psychological aetiologies [26]. Normal sexual function is a product of many factors and these factors include neural activity, vascular events, intracavernosal nitric oxide system and androgen [27]. Thus malfunction of any of these could lead to sexual dysfunction which refers to repeated inability to achieve normal sexual intercourse, it can also be viewed as disorders that interfere with the full sexual response cycle [28]. Male sexual dysfunction is further classified as a disorder of desire, that is persistent absence of sexual fantasy and need for sex, erectile dysfunction, that is the repeated incapability to develop and sustain a penile erection for intercourse and ejaculation in 50% or more of attempts [28,29], disorder of ejaculation that is problem with expulsion of semen at the climax of the sexual act, disorder of orgasm that is delay in or absence of orgasm after a normal sexual excitement phase during sexual activity [30], failure to detumescence that is prolonged (> 4 hours duration) and extreme painful erection unaccompanied by sexual desire [31]. The causes of male sexual dysfunction include factors like psychological disorders (performance anxiety, strained relationships, depression, stress, guilt, fear of sexual failure), hormonal condition (androgen deficiency, hyperprolactinemia), chronic medical conditions (diabetes, hypertension), penile disease (priapism, smooth muscle dysfunction), neurological disorder (Parkinson disease, stroke, cerebral trauma, alzheimer's disease, spinal cord or nerve injury), drug side effect (antihypertensive, psychiatric medications, antiulcer and antidepressants), lifestyle (chronic alcohol abuse, cigarette smoking), aging and systemic disease (cardiac, hepatic, renal, pulmonary, metabolic), post organ transplant [31,32,33].

Despite the medical advancements in treatment and treatment facilities for male sexual dysfunction [35], most sufferers often shy away from these treatment options and these could be accredited to its sensitivity and the social stigma attached to male sexual dysfunction in African [36], these treatment options are equally very expensive [37,38], not easily accessed by the poor and rural dwellers

[39,40]and are often associated with some serious side effects[41], consequently medicinal plants with marked pharmacological activities[42,43] are freely available all through the year [44], cheap and often with little or no toxicity[45,46,47] and are being explored globally as panacea. All through history many preparations from plants have been used and reputed to have sex invigorating (aphrodisiac) properties and these include Yohimbine, Gingseng, *Massularia acuminata*, *Montanoa tomentosa* [18,48].The individual and composite ethanolextract of the *Carpolobia lutea* and *Sabiceacalycina* are two of such local aphrodisiacs that is essentially used by the people of Niger Delta region of Nigeria, but with little or no scientific traction.The plant *C. lutea* G. Don (Polygalaceae) is a small tree that grows to about 15ft high and it is extensively distributed across the rainforest of tropical Africa. It is commonly called cattle stick. Ethnopharmacologically, different parts of the plant have been harnessed in the treatment and management of numerous health challenges. For example, the leaves are used as anti-pyretic, remedy for ulcer, malaria, dermal infection, venereal diseases, sterility, vermifuge, taenifuge, stomach problem, diarrhoea, headache, leprosy, snakebite and wounds[49,50]. The leaves have also been used to promote child birth while the root bark have been implicated for treating rheumatism, general pain and insanity. The stem bark is dried and taken as snuff to cure migraine. Moreso the decoction of the root is reputed in Southern Nigeria as a sex invigorating drink[51].*S.calycina* belongs to the Rubiceae family. Its decoction in the region is used as a laxative. The mashed leaves are applied topically to the limbs of small children to fortify their bones and to assist them to walk. The mashed leaves are also used to treat wounds[52]. The infusion is also believed to enhance memory and to manage senile dementia[53].The present study was therefore to appraise the sex restoration and invigorating properties of the composite ethanol extract of the *C. lutea* and *S. calycina* at a dose of 50, 100 and 200 mg/kg body weight of albino rats environmentally challenged withBonny light crude oil (BLCO).

2.0 Materials and Methods

2.1 Plant Source and Authentication

The stem of *S.calycina*and *C. lutea* were harvested in Odi town, KolokumaOpokuma Local Government Area of Bayelsa State, Nigeria. Identification and authentication was done at the Plant Science and Biotechnology Department, Ekiti State University, Ado Ekiti, Ekiti State, Nigeriawith a voucher specimen numbers given as UHAE2019808 and UHAE2019809 respectively and the specimen were kept in the herbarium for future reference.

2.2 Preparation of Composite of *S.calycina*and *C. lutea* Extract

The stem of the plant material (*S.calycina* and *C. lutea*) were carefully washed with distilled water to remove sand and other impurities, the samples was then shade dried so as to give a constant weight,

and subsequently milled into fine powder using an electric blender (Binatone Blender/Grinder BLG-595). 200 g of the powdered *S. calycina* and *C. lutea* (i.e. 100 g each of *S. calycina* and *C. lutea*) was extracted in 600 mL of absolute ethanol for 24 h at room temperature with constant shaking using a flask shaker (Model, Denly A - 500). The extract was filtered with Whatman No 1 filter paper and the resulting filtrate evaporated to dryness using a Rotatory evaporator at 37°C to give 5.23 g, the resultant concentrate was then reconstituted using distilled water to give the required working doses used in the study.

2.3 Animals Used for the Study:

Forty healthy, sexually experienced male albino rats (*Rattus norvegicus*) weighing between 120-135g were obtained from the Animal House unit of the Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria. The animals were kept in separate investigational room, which was clean and well-ventilated at temperature between 28-30°C, under natural dark/light cycle with free access to standard rat pellets and water *ad-libitum* during the period of acclimatization which lasted for one week and the experimental period which lasted for 21 days. Animals for the research were handled in accordance with the Institutional Animal Ethics Committee (IAEC) as per guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals. Protocols for the use of these animals was approved by the Directorate of Research and Quality Assurance, Federal University Otuoke, Bayelsa State via an approval DRQA/FUO/0100/13/12/23.

2.4 Experimental Design

2.4.1 Male rats:

The forty rats were randomly divided into two groups (A and B) of twenty rats each.

The group A, Non-Bonny Light Crude Oil Environmentally challenged rats (NBLCO) were further subdivided into four groups and subjected to the following experimental regimen below.

Group A₁ Non Crude Oil Contaminated Environment (NBLCO)+ 50 mL of distilled water.

Group A₂ Non Crude Oil Contaminated Environment(NBLCO) +50mg/kg of composite extract

Group A₃ Non Crude Oil Contaminated Environment(NBLCO) + 100mg/kg of composite extract

Group A₄ Non Crude Oil Contaminated Environment(NBLCO)+ 200mg/kg of composite extract

The group B, Bonny Light Crude Oil Environmentally challenged rats (BLCO) were further subdivided into four groups and subjected to the following experimental regimen below.

Group B₁ Crude Oil Contaminated Environment(BLCO) + 50 mL of distilled water.

- Group B₂ Crude Oil Contaminated Environment(BLCO) + 50mg/kg of composite extract
- Group B₃ Crude Oil Contaminated Environment (BLCO)+ 100mg/kg of composite extract
- Group B₄ Crude Oil Contaminated Environment (BLCO)+ 200mg/kg of composite extract

2.42 Crude Oil Contaminated Environment Simulation

The crude oil contaminated environment was simulated in a section of the Animal House, 20 mL of Bonny light crude oil was spilled homogeneously on the beddings of the metabolic cages measuring 40 cm × 30 cm × 20 cm housing the animals used for the crude oil environmentally challenged groups. The animals were housed in these challenged environment for 3 weeks.

2.43 Female rats

The fifty female rats used for mating behavior test were artificially brought to estrus (heat) this is because the female allow mating only during the estrus phase, and this was done by administering estradiol benzoate 10µg/100g orally 48 h prior to mating and progesterone injected subcutaneously at a dose of 0.5 mg/100g, 6 h prior to mating [20].

2.5 Method Extract Administration

The various doses of the crude extracts i.e. used (50, 100 and 200 mg/kg) were administered orally by using oropharyngeal cannula to all rats in the different groups for three weeks (21 days)

2.6 Mating Behavioural Study

The mating behavior study was carried out on the 22nd day after the initiation of the administration of the extracts by adopting the methods of [20,48]. The experiments were conducted between 19:00 hr and 22:00 hr in the same animal house and under the light of the same intensity, so as to avoid testing the rats in an unacquainted condition and environment. The responsive female was presented to the cage of the male measuring 40 cm x 30 cm x 20 cm. The receptive female and male were viewed from the cage side for proceptive, precopulatory and copulatory behaviours. The test was terminated if the male failed to display sexual interest, and if the female did not display receptivity she was also substituted with another artificially warmed female. The sequence of events and phases of mating was monitored for 30 min observatory period using a stop watch, and the following male sexual behavior indices recorded and calculated.

2.61 Mount Latency (ML)

This is the time interval between the introduction of the female to the first mount by the male [20,54]. A mount is when the male rat straddles the female from the back and clasps her flanks with his front feet.

2.62 Mount Frequency (MF)

The number of times the male donned the copulatory position without achieving intromission often typified by raising of the male's fore-body over the hindquarters of the female and clasping her flanks with his paw. There is however, no vaginal penetration (Yakubu et al., 2014).

2.63 Intromission Latency (IL)

The time interval from the introduction of the female until the first intromission of the female (vaginal penetration) by the male, it is typically exemplified by pelvic thrusting and pouncing dismount [20,48]

2.64 Intromission Frequency (IF)

The number of intromissions (vaginal penetration) made by the male from the first time of introduction of the female [20,48].

2.65 Ejaculatory Latency (EL)

The time interval between the first intromission and ejaculation is often typified by longer, deeper pelvic thrusting and slow dismount, followed by a period of reduced activity [20,48]

2.66 Ejaculation Frequency (EF)

The number of times there was discharge of semen by the males after vaginal penetration typified by rhythmic contraction of the posterior abdomen [20,48].

2.67 Post Ejaculatory Interval (PEI)

The time interval from ejaculation to intromission of the next mating series [20,48].

2.68 Copulatory Efficiency (CP)

$$CP = \frac{\text{number of intromissions}}{\text{number of mounts}} \times 100 [20,48]$$

2.7 Biochemical Analysis

2.71 Method of Blood Collection

24 hrs after extract dosing and mating behavioral test, the animals were anaesthetized in a jar containing cotton wool soaked in chloroform. On anaesthetization of the animals, blood samples were collected via cardiac puncture into plain sample bottles. The blood samples were then allowed to clot

for 15 min at room temperature and subsequently centrifuged at 2000 rpm for 10 min with centrifuge (model CE-800) and the sera collected and used for hormonal assay.

2.72 Assay Kits and drugs

Testosterone, follicle stimulating hormone (FSH), luteinizing hormone, prolactin and progesterone radioimmunoassay test kits are products of BYK-SangticDiagnostica, GmbH and Co. KG, while estradiol benzoate and progesterone are products of Sigma Chemicals, St. Louis, USA and Shalina Laboratories, Mumbai, India, respectively.

2.73 Hormonal Assay

Serum samples were assayed for the following hormones testosterone, follicle stimulating hormone, luteinizing hormone, prolactin and progesterone by using the procedure described by BYK-SangticDiagnostica. This was based on the principle of radioimmunoassay of competitive binding between the sample serum and the standards for a constant amount of the antisera [55].

2.8 Data Analysis

The Statistical level of significance was considered to occur at 95% ($p < 0.05$). Data analysis was piloted with the aid of the Statistical Package for the Social Sciences (SPSS) version 21. The one-way analysis of variance (ANOVA) followed by the Tukey post hoc test was used to examine the data. Results were expressed as mean \pm standard error of the mean. The charts were plotted using Graphpad Prism 8.

3.0 Results

3.1 Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behaviour (Mount latency, Intromission latency, Ejaculatory latency and Post ejaculatory interval) of both NBLCO and BLCO Environmentally Challenged Albino Rat

The consequences of the combined extract of *S. calycina* and *C. lutea* of the respective study doses (50, 100 and 200 mg/kg) on mating behavioural indices (Mount latency, Intromission latency, Ejaculatory latency and Post ejaculatory latency) of Non-Bonny Light Crude Oil Challenged Albino Rat are shown in Fig. 1.0A. and that of the Bonny Light Crude Oil Challenged Albino Rat are shown in Fig. 1.0B. The mount latencies, intromission latencies and post ejaculatory intervals showed dose-dependent decreases but with the 100 and 200 mg/kg been statistically significant ($p < 0.05$). The ejaculatory latencies showed dose-dependent increases but increases caused by only the 100 and 200 mg/kg are statistically significant ($p < 0.05$).

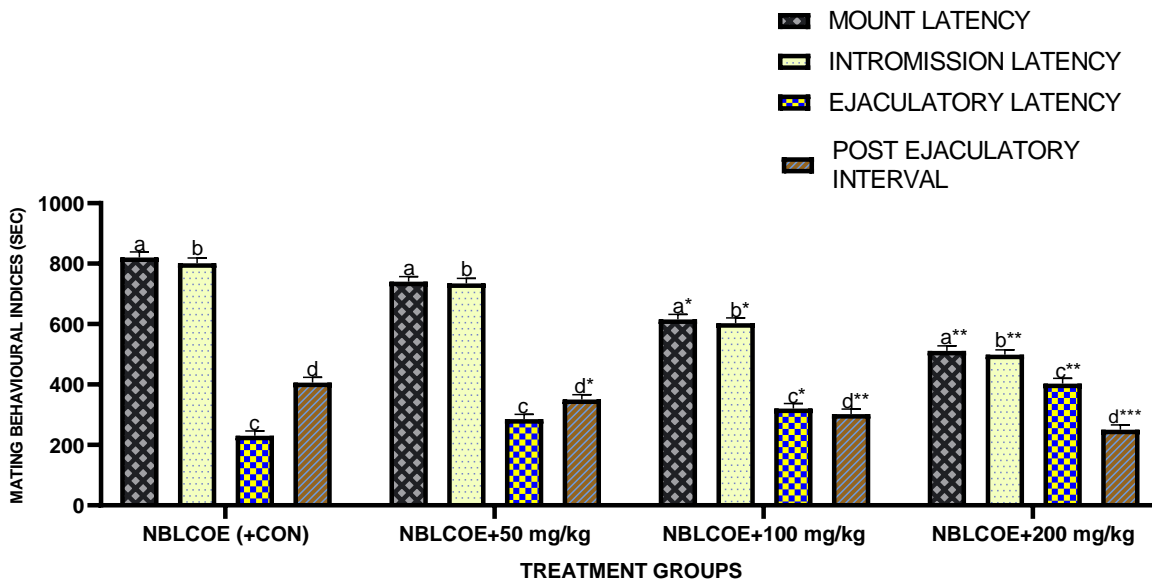


Fig. 1.0A: Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behavioural test (Mount latency, Intromission latency, Ejaculatory latency and Post ejaculatory interval) of NBLCOE environmentally challenged albino rats. Data are Mean \pm SD (n=5) mean value of parameters with same superscript letter but with asterisk are statistically different ($p < 0.05$), one-way Analysis of Variance (ANOVA) followed by Post-hoc Tukey.

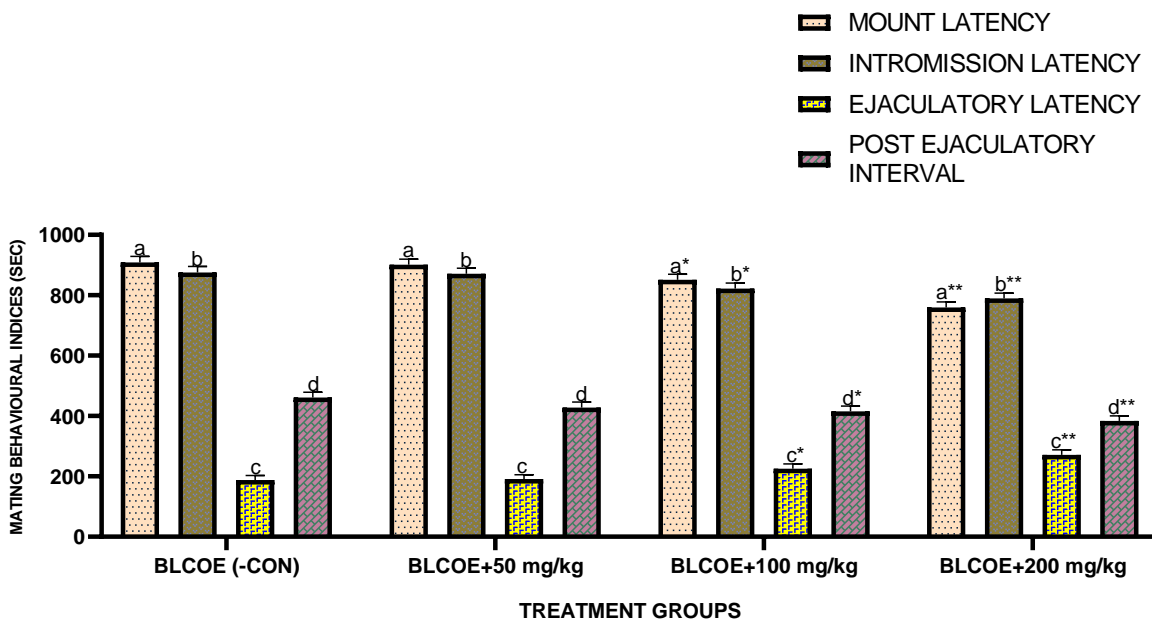


Fig. 1.0B: Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behavioral test (Mount latency, Intromission latency, Ejaculatory latency and Post ejaculatory latency) of BLCOE environmentally challenged albino rats. Data are Mean \pm SD (n=5) mean value of parameters with same superscript letter but with asterisks are statistically different ($p < 0.05$), one-way Analysis of Variance (ANOVA) followed by Post-hoc Tukey.

3.2 Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behaviour (Mount frequencies, Intromission frequencies and Ejaculatory frequencies of both NBLCO and BLCO Environmentally Challenged Albino rats.

The influence of the combined extract of *S. calycina* and *C. lutea* of the respective study doses (50, 100 and 200 mg/kg) on mating behavioural indices of Mount frequencies, Intromission frequencies and Ejaculatory frequencies of NBLCO Challenged Albino Rat are shown in Fig. 2.0A. and that of the BLCO Challenged Albino Rat are shown in Fig. 2.0B. The mount frequencies, intromission frequencies and ejaculatory frequencies all showed dose-dependent increases, however, only the 50 mg/kg dose did not exhibit statistically significant ($p < 0.05$) increases.

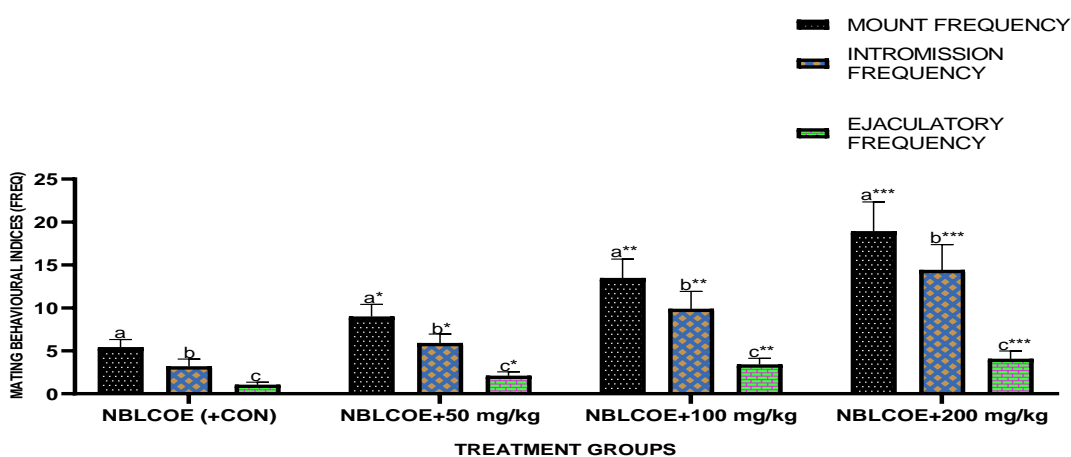


Fig. 2.0A: Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behavioural test (Mount frequency, Intromission frequency and Ejaculatory frequency) of NBLCO environmentally challenged albino rats. Data are Mean \pm SD (n=5) mean value of parameters with same superscript letter but with asterisks are statistically different ($p < 0.05$). one-way Analysis of Variance (ANOVA) followed by Post-hoc Tukey.

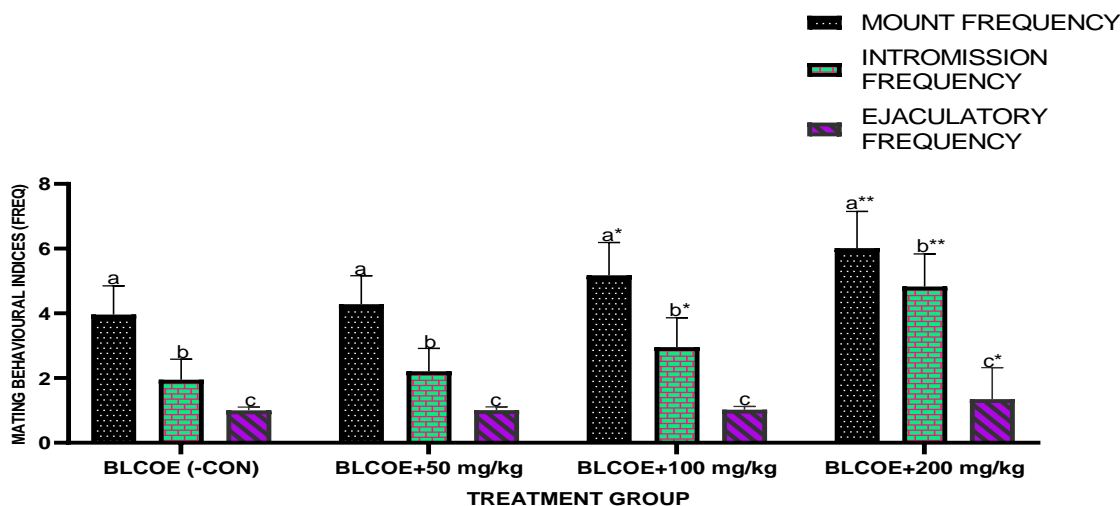


Fig. 2.0B: Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behavioural test (Mount frequency, Intromission frequency and Ejaculatory frequency) of BLCO environmentally challenged albino rats. Data are Mean \pm SD (n=5) mean value of parameters with same superscript letter but with asterisks are statistically different ($p < 0.05$). one-way Analysis of Variance (ANOVA) followed by Post-hoc Tukey.

3.3 Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Copulatory efficiency on NBLCO and BLCO Environmentally Challenged Albino Rats.

The potency of the combined extract of *S. calycina* and *C. lutea* on the Copulatory efficiency of the respective study doses of (50, 100 and 200 mg/kg) on both the NBLCO and BLCO environmentally challenged albino rat is presented in Fig. 3.0. Statistically significant increases ($p < 0.05$) was observed in all the NBLCO challenged groups, while only the 200 mg/kg had significant increase ($p < 0.05$) on Copulatory efficiency.

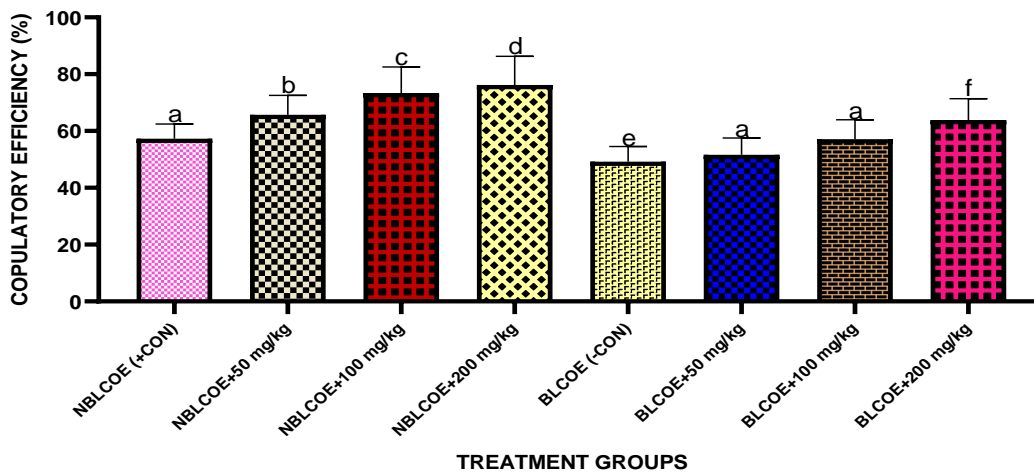


Fig. 3.0: Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Copulatory efficiency test of both NBLCO and BLCO environmentally challenged albino rats. Data are Mean \pm SD (n=5) mean value of parameters with different superscript letter are statistically different ($p < 0.05$), one-way Analysis of Variance (ANOVA) followed by Post-hoc Tukey.

3.4 Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Reproductive (sex hormones) levels of both NBLCO and BLCO Environmentally Challenged Albino Rat

The influence of the combined extract of *S. calycina* and *C. lutea* of the respective study doses (50, 100 and 200 mg/kg) on Reproductive hormonal levels (testosterone, prolactin, luteinizing hormone, follicle stimulating hormone and progesterone) on NBLCO challenged albino rat is shown in Fig. 4.0A. and that of the BLCO environmentally challenged albino rat is shown in Fig. 4.0B. In the NBLCO groups, all doses of the extract significantly increased ($p < 0.05$) the levels of testosterone, luteinizing hormone, follicle stimulating hormone and progesterone while significantly decreasing ($p < 0.05$) the prolactin concentration. However, in the BLCO challenged group all doses also significantly increased ($p < 0.05$) the concentrations of testosterone, luteinizing hormone, follicle stimulating hormone and progesterone while significantly decreasing ($p < 0.05$) the prolactin concentration except the 50 mg/kg.

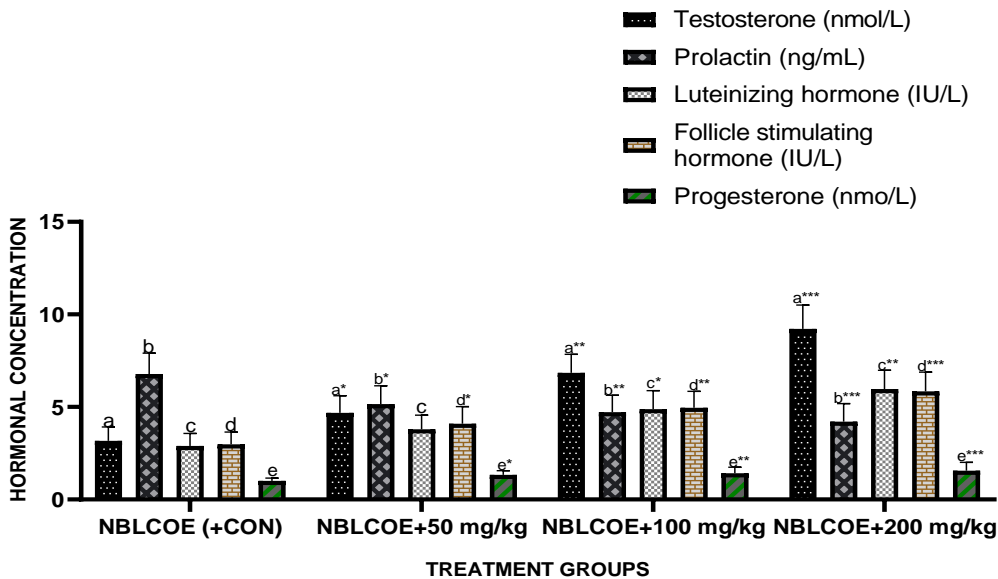


Fig. 4.0A: Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Reproductive Hormonal levels of NBLCO environmentally challenged albino rats. Data are Mean \pm SD (n=5) mean value of parameters with same superscript letter but with asterisks are statistically different ($p < 0.05$). one-way Analysis of Variance (ANOVA) followed by Post-hoc Tukey.

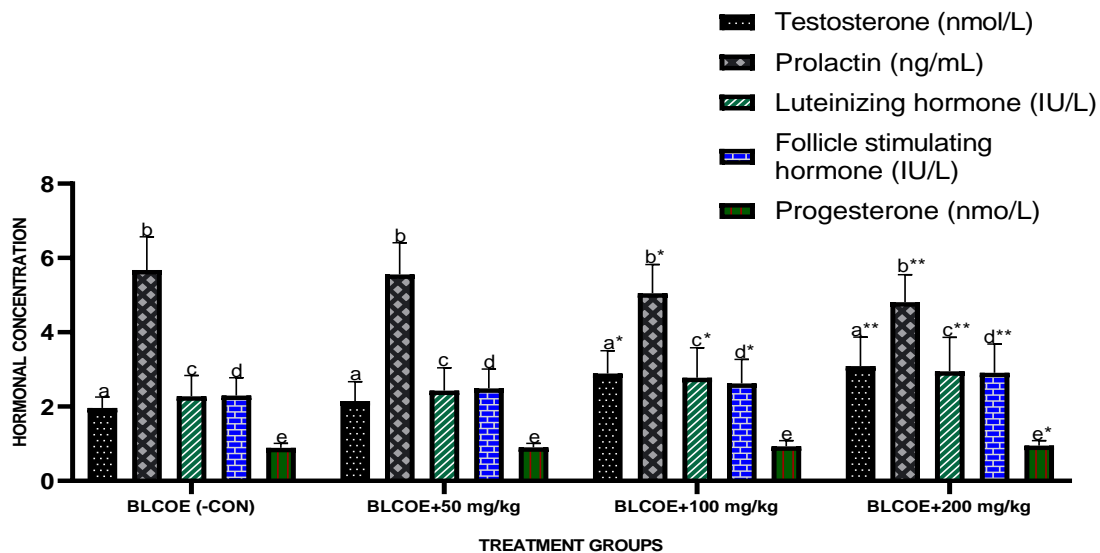


Fig. 4.0B: Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Reproductive Hormonal levels of BLCO environmentally challenged albino rats. Data are Mean \pm SD (n=5) mean value of parameters with same superscript letter but with asterisks are statistically different ($p < 0.05$), one-way Analysis of Variance (ANOVA) followed by Post-hoc Tukey.

4.0 Discussion

Outside the financial remunerations accruable from crude oil, the effects of the exploration and exploitation of crude oil results in the pollution of the environment caused by spillage, causes of the spillage include but not limited to operational errors, equipment failure and vandalism and activities of illegal refiners (sabotage). The crude oil upon contact with the environment undergo rapid

modifications caused by sundry chemical reactions, biochemical transformation and microbial degradation forming products that are often more noxious than the original crude oil in its itself. Crude oil and its transformation products are carcinogenic, neurotoxic, endocrine disrupting and myriads of other toxicological effects [56].

4.1 Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behavioural indices of Mount latencies, intromission latencies, ejaculatory latencies, post ejaculatory intervals, Mount frequencies, Intromission frequencies and Ejaculatory frequencies of both NBLCO and BLCO Environmentally Challenged Albino Rats.

Both Mount latency and intromission latency are biomarkers of sexual drive, desire or libido, there is usually an inverse correlation between mount latency, intromission latency and sexual motivation. The BLCO environmentally challenged rat had significantly higher ($p < 0.05$) mount and intromission latency, this can be correlated to poor sexual appetite probably arising from endocrine disruption [56] pathway for the synthesis of sex hormones like testosterone, follicle stimulating hormones and luteinizing hormones [57]. The extract was able to significantly decrease ($P < 0.05$) the mount latency and intromission latency to a value not significantly different ($p > 0.05$) from the NBLCO challenged rats and this might infer restoration of sexual appetite and arousal probably occasioned by the positive stimulatory effect of the extract on the nitric oxide synthase enzyme [58].

Mount and intromission frequencies are pointers of sexual vigour, stamina, power and energy. Sexual activity itself involves energy expenditure [59]. The significant increase ($p < 0.05$) in these factors may be credited to energy generation and utilization potentials of phytonutrients inherent in these plant samples [58,60].

Ejaculatory latency and ejaculatory frequency are pointers of enhanced sexual performance, the extract was also able to significantly increase ejaculatory latency and ejaculatory frequency ($P < 0.05$). Ejaculatory latency also infers extended coitus interval which deciphers increased staying power, strength and vigour thus corroborating its sexual boasting potentials just other aphrodisiac plants that have been studied e.g. *Dactylorhiza hatagirea* and *Mondia whitei* [18]. The significantly increased period of ejaculatory latency may be ascribed to the presence of various flavonoids in both plant extract with the ability to upsurge the concentration level of cGMP and, therefore, induce vascular smooth muscle relaxation while also inhibiting the activity of PDE-5 [61,62]. The overall effect of this process is to amplify the blood influx into the penile tissue and thus resulting in an erection of the penis and subsequent delay in ejaculation that leads to detumescence [63].

The post ejaculatory interval is a veritable marker of sexual power, libido and a fast speed of recovery from fatigue after the initial succession of mating. All doses of the extract significantly decreased the post ejaculatory interval ($P < 0.05$) in the NBLCO group but only the 100 and 200 mg/kg was able to significantly decrease the post ejaculatory interval in the BLCO challenged group and compared favourably with other aphrodisiac plants that have been studied e.g. *Chlorophytum boriviliaunum*[64] and *Syzygium aromaticum*[65].

The copulatory efficiency is a sign that the sexual act of the male was well boosted with well-coordinated pelvic thrusting and this further goes to buttress sustained increase in interest, attention, dexterity and stamina in the sexual act [66]. All doses of the extract were able to significantly increase ($P < 0.05$) the copulatory efficiency in the NBLCO group while only the 200 mg/kg was only able to significantly increase ($P < 0.05$) the copulatory efficiency in the BLCOE challenged rats. Thus lending credence to its aphrodisiac potency which compares to other aphrodisiac plants that have been studied e.g. *Alpinia calcarata*[67] and *Withaniasomnifera*[68].

The precopulatory and copulatory physiognomies of the extract treated rats showed that the rats were exceptionally aroused and the effects of the extract on the mating behaviour were dose dependent with the 200 mg/kg body weight been more effective.

4.2 Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Reproductive (sex hormones) levels of both NBLCO and BLCO Environmentally Challenged Albino Rat

Testosterone is produced by the laryngeal cells of the testicles, it is a sex hormone and the most vital androgen that plays fundamental roles in the body. In men, it regulates sex drive often referred to as libido, bone mass, fat distribution, muscle mass and strength, and the production of sperm [69]. A normal level of testosterone sustains the male secondary sex characteristics and normal sexual desire, which stimulates sperm maturation and protein synthesis, specifically in reproductive organs and muscles [70]. Androgen deficiency is a primary contributory factor for many generic clinical diseases and may result to one form of sexual dysfunction or the other and a decrease in reproductive ability in men [70]. FSH is secreted by the Sertoli cells, plays an essential function in testicular development. FSH also help to maintain the testosterone concentration in spermatogenic cells, stimulates the binding of androgen binding protein (ABP) to testosterone, and normalizes the numbers of spermatogenic cells [70]. LH are also required for safeguarding testosterone levels, hence an increase in LH and FSH inevitably causes an upsurge in testosterone levels [22]. Testosterone supplementation helps to improve sexual function and libido [71,72], alongside the magnification of orgasm intensity [73]. The concentrations of testosterone, FSH and LH are often used as an indirect indicator for appraising male

sexual function. The likely incapability of the pituitary gland to maintain the ratio of these hormones may feasibly upset numerous processes involved in sexual function. High concentration of prolactin in men (hyperprolactinemia) has been associated with hypogonadism, reduced sperm count and motility, erectile dysfunction and decreased libido [70]. The positive stimulatory capacity of the extract at the respective doses are primarily due to their bioactive ingredients [73], these bioactive compounds exert positive stimulatory effects on sex/reproductive hormones, this is evident by the positive effect on the respective mating behavioral indices.

5.0 Conclusion

Findings from this study has shown that rats challenged with BLCO had statistically significant reduced sexual function as depicted by the mating behavioural test and reduced testosterone, FSH, LH and PRG, administration of the combined ethanol extract of *S. carlycina* and *C. lutea* administered at a dose level of 100 and 200 mg/kg was able to restore and invigorate sexual potency. All doses of the extract were also able to heightened sexual functions in the NBLCO rats.

References

1. Abubakar MY, Ahmad SS, Sani SB, Jinjiri, AY. Analysis of the Impact of Oil Revenue on the Nigerian Economy. IOSR Journal of Economics and Finance (IOSR-JEF). 2016; 7(4):10-21
2. UNDP-Project. Niger Delta Biodiversity Project. [www.undp.org.project.NGA](http://www.undp.org/project.NGA). 2012; Accessed on 14/02/16.
3. Ordinioha B, Brisibe S. The Human Health Implications of Crude Oil Spills in the Niger-Delta Nigeria: An interpretation of published studies. Nigerian Medical Journal. 2013; 54 (1):10-16.
4. Idoniboye BE, Andy AJ. Effect of oil pollution on aquatic environment. In Proceedings of 1985 Seminar on Petroleum Industry and the Nigerians Environment; 1985.
5. Teddy C. Adias, Anslem O. Ajugwo, Tosan Erhabor, and Clement U. Nyenke, "Effect of Pumpkin Extract (*Telfairia occidentalis*) on Routine Haematological Parameters in Acetone-Induced Oxidative Stress Albino Rats." American Journal of Food Science and Technology. 2013; 1(4): 67-69. doi: 10.12691/ajfst-1-4-1
6. Igwe FU, dokubo A, Ukpaka CP. Overview of Crude Oil Toxicity: A Potential Health and Environmental Hazard in the Niger Delta. Nigerian Journal of Oil and Gas Technology. 2016; 2(2):94-104
7. Adeyemi O, Adeyemi O. Evaluation of toxic effect of oral co-administration of crude oil and vitamin C on antioxidant system of albino rats. Biokemistri. 2021; 33(4):227-233

8. Adesanya OA, Shittu LA, Omonigbehin EA, Tayo AO. Spermatotoxic Impact of Bonny Light Crude Oil (BLCO) ingestion on adult male Swiss albino mice. *International Journal of Physical Sciences*. 2009; 4:349-353
9. Oruambo IF, Peter-Omonighia CA. Alterations in chromatin DNA and protein absorbance ratio in the liver of albino rats (*Rattus norvegicus*) treated with Bonny Light Crude Oil. *Journal of Applied Biosciences*. 2012; 54:3911-3915
10. Raji Y, Hart VO. Influence of prolonged exposure to Nigerian Bonny light crude oil on fertility indices in rats. *Nigerian J Physiological Sciences*. 2012;27(1):55-63.
11. Jeje SO, Ukwenya VO, Anita AB, Daramola OM. Maternal exposure to Bonny Light Crude Oil Altered Reproductive indices in Male and Female offspring of Wistar rats. *Nigerian Journal of Physiological Sciences*. 2021;36(1):49-55.
12. Ajonuma LCC, Fapohunda DO, Bamiro SA, Afolabi D, Abokede N, Giwa KT. Prolonged Ingestion of Crude Oil Spill Contaminated Water Affects Male Reproductive System, Hormones and Accessory Organs in Wistar rats. *Fertility and Sterility*. 2023; 120(4): supplement E130. DOI:<https://doi.org/10.1016/j.fertnstert.2023.08.405>
13. Kaiser FE. *The medical clinics of North American: the aging male patient. Erectile dysfunction in the aging man*. 1999. W.B Saunder, P.A
14. Lew-Starowicz Z, Czajkowska K. Prevalence of sexual dysfunctions and associated risk factors in Poland. *Archives of Medical Science*. 2022;18(4):1031–1040. DOI: <https://doi.org/10.5114/aoms.2019.86794>
15. Lauman EO, Paik A, Rosen RC. Sexual dysfunction in the United States, Prevalence and Predictors. *Journal of the American Medical Association*. 1999; 281:537–544.
16. Idung AU, Abasiubong F, Ukott IA, Udoh SB, Unadike BC. Prevalence and risk factors of erectile dysfunction in Niger delta region, Nigeria. *African Health Sciences*. 2012; (2):160-165.
17. Umuerri EM, Ayandele CO. Erectile dysfunction and premature ejaculation in Nigerians. *Highland Medical Research Journal*. 2021; 21(2):24-30
18. Baljinder S, Vikas A, Parveen B, Ranjit S, Dharmendra K. Pharmacological Potential of Plants used as Aphrodisiacs. *International Journal of Pharmaceutical Sciences Review and Research*. 2010; 5(1):104-113.
19. Kpomah ED. Biochemical effects of *Zanthoxylumleprieurii* Guill & Perr on reproductive hormones, liver function and plasma enzyme activity of male wistar rats. *International Journal of Basic Science and Technology*. 2019; 5(2):73-78
20. Kpomah ED, Uwakwe AA, Abbey BW. Aphrodisiac studies of diherbal mixture of *Zanthoxylum leprieurii* Guill. & Perr. and *Piper guinense* Schumach. & Thonn. on male Wistar

- rat. *Global Journal of Research on Medicinal Plants and Indigenous Medicine*. 2012; 1 (9):381-390
21. Ramlachan P, Campbell M.M. Male sexual dysfunction. *South Africa Medical Journal*. 2014; 104(6):447
 22. Yakubu MT, Akanji MA, Oladiji AT. Male sexual dysfunction and methods used in Assessing Medicinal Plants with Aphrodisiac Potentials. *Pharmacognosy Review*. 2007; 1(1):49-56.
 23. Kolodny RC, Masters WH, Johnson VE. Sexual Anatomy and Physiology. In *Textbook of Sexual Medicine*. Little Brown and Co., Boston. 1979; M. A. Pp. 1-28.
 24. Govier FE, Asase D, Hefty TR, McClure RD, Pritchett TR, Weissman RM. Timing of penile color flow duplex ultrasonography using a triple drug mixture. *Journal of Urology*. 1995; 153: 1472-1475.
 25. Walsh PC, Wilson, JD. (1987). Impotence and Infertility in Men. In: Braunwald, E., Isselbacher, K.J. Petersdorf, R.S., Wilson, J.D., Martins, J.B., Fauci A.S. (Eds). *Harrison's Principle of Internal Medicine* McGraw-Hill Book Co. New-York N.Y. Pp. 217 – 220.
 26. Hawton, K. (1985). *Sex Therapy: A practical Guide*. Oxford University Press. New York, NY, Pp. 123-199.
 27. Anderson D, Laforge J, Ross MM, Vanlangendonck R, Hasoon J, Viswanath O, Kaye AD, Urits I. Male Sexual Dysfunction. *Health Psychology Research*. 2022; 20;10(3):37533. doi: 10.52965/001c.37533. PMID: 35999971; PMCID: PMC9392840.
 28. Lei C, Guang-rui S, Dan-dan H, Yang L, Chen-chao M, Min S, Bin-xiao S, Guang-jiang S. Male sexual dysfunction: A review of literature on its pathological mechanisms, potential risk factors, and herbal drug intervention. *Biomedicine & Pharmacotherapy*. 2019; <https://doi.org/10.1016/j.biopha.2019.01.046>.
 29. Kandeel FR, Vivien KTK, Ronald SS. Male Sexual Function and its Disorder: Physiology, Pathophysiology, Clinical Investigation and Treatment. *Endocrine reviews*. 2001; 22(3):342-388
 30. Marais A. The pharmacological management of erectile dysfunction. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*. 2015; 20(3):4-8 <http://dx.doi.org/10.1080/16089677.2015.1056468>
 31. Chung E. Sexuality in Ageing Male: Review of Pathophysiology and Treatment Strategies for Various Male Sexual Dysfunctions. *Medical Sciences (Basel)*. 2019;7(10):98. doi: 10.3390/medsci7100098. PMID: 31547182; PMCID: PMC6835548.
 32. Souza Júnior EV, Souza CS, Silva Filho BF, Siqueira LR, Silva CS, Sawada NO. Sexual function positively correlated with older adults' sexuality and quality of life. *Revista Brasileira de Enfermagem*. 2022; 75(4):1-10. <https://doi.org/10.1590/0034-7167-2021-0939 e20210939>

33. Majzoub A, Elbardisi H, Madani S, Leisegang K, Mahdi M, Agarwal A, Henkel R, Khalafalla K, ElSaid S, Arafa M. Impact of body composition analysis on male sexual function: A metabolic age study. *Frontiers in Endocrinology*. 2023; 13:1050441. doi: 10.3389/fendo.2022.1050441
34. Takure AO, Adebayo SA, Okeke LI, Olapade-Olaopa EO, Shittu OB. Erectile Dysfunction Among Men Attending Surgical Outpatients Department in a Tertiary Hospital in South-Western Nigeria. *Nigerian Journal of Surgery*. 2016; 22(1):32-6. doi: 10.4103/1117-6806.176398. PMID: 27013856; PMCID: PMC4785689.
35. Muhammad AZ, Grema BA, Shuaibu A, Michael GC. Prevalence, severity, and correlates of erectile dysfunction among male adult patients of a primary care clinic in North-West Nigeria. *African Health Sciences*. 2023;23(2):670-81. <https://dx.doi.org/10.4314/ahs.v23i2.77>
36. Hackett G, Kirby M, Ramachandran S. (2020). Erectile dysfunction: is the NHS men's health friendly? *Trends in Urology and Men's Health*. 2020; 11(5):6-12
37. Bell C, Hadi MA, Khanal S, Paudyal V. Prescribing patterns and costs associated with erectile dysfunction drugs in England: a time trend analysis. *BJGP Open*. 2021; 5(2):bjgpopen20X101145. doi: 10.3399/bjgpopen20X101145
38. Rodler S, von Büren J, Buchner A, Stief C, Elkhanova K, Wülfing C, Jungmann S. Epidemiology and Treatment Barriers of Patients with Erectile Dysfunction Using an Online Prescription Platform: A Cross-Sectional Study. *Sexual Medicine*. 2020; 8(3):370-377doi: 10.1016/j.esxm.2020.04.001.
39. Pretorius D, Couper ID, Mlambo MG. Neglected sexual dysfunction symptoms amongst chronic patients during routine consultations in rural clinics in the North West province. *African Journal of Primary Health Care and Family Medicine*. 2021;13(1), a2850. <https://doi.org/10.4102/phcfm.v13i1.2850>
40. Kaplan-Marans E, Sandozi A, Martinez M, Lee J, Schulman A, Khurgin J. Medications Most Commonly Associated with Erectile Dysfunction: Evaluation of the Food and Drug Administration National Pharmacovigilance Database. *Sexual Medicine*. 2022;10(5):100543. doi: 10.1016/j.esxm.2022.100543. Epub 2022 Jul 14. PMID: 35843193; PMCID: PMC9537247.
41. Kpomah ED, Onyeike EN, Kpomah B. Evaluation of some Elemental, Bioactive Compounds and Proximate Composition of three commonly used Herbal Plants in the Niger Delta Region of Nigeria. *Chemistry Research Journal*. 2018; 3(2):12-21
42. Kpomah ED, Monday DA. Kpomah, B. GCMS analysis of leaves and seeds of *Piper guineense* Schumacher & Thoon. *African Scientist*. 2019; 20(3):127-138

43. Kpomah ED, Odokwo EO. Comparative Phytochemical, Proximate and Some Mineral Composition of the Leaves and Stem Bark of *Spondiamombin* (*L. anacardiaceae*). Annual Research & Review in Biology. 2020; 35(6): 90-98
44. Kpomah ED, Arhoghro EM. Effects of doses of *Bryophylumpinnatum* and glibenclamide on serum glucose and lipid profile in alloxan-diabetic rats. Indian Journal of Drugs and Disease. 2012; 1 (5):124-128
45. Kpomah ED, Ogbogbo J, Kpomah B. Sub-acute toxicity studies of *Phyllanthus amarus* on haematological parameters and some plasma enzyme activities in mice. International journal of basic science and technology. 2017; 3(1):53-58
46. Kpomah ED. Kpomah B. Microstructural Tissue Assessment, Sex Hormones and Biochemical Investigations following Acute Administration of *Piper guineense* Schumach & Thonn. on Female *Rattus norvegicus*. IOSR Journal of Applied Chemistry. 2018; 11(5):9-17
47. Yakubu MT, Akanji MA. Effect of aqueous extracts of *Massularia acuminata* stem on sexual behaviour of male wistar rats. Evidence based complementary and Alternative Medicine. 2011; retrieved <http://www.hindawi.com/Journals/ecam/2011/738103.htm>.17/2/2011.
48. Ajibesin KK, Ekpo AB, Bala DN, Essien EE, Adesanya SA. Ethnobotanical survey of Akwa Ibom State of Nigeria. Journal of Ethnopharmacology. 2007; 115: 387-408.
49. Akpan MM, Okokon JE, Akpan JE. Antidiabetic and hypolipidemic activities of ethanolic leaf extract and fractions of *Carpolobia lutea*. Molecular clinical pharmacology. 2012;3:100-107
50. Yakubu MT, Jimoh RO. *Carpolobia lutea* roots restore sexual arousal and performance in paroxetine-induced sexually impaired male rats. Revista International de Andrologia. 2014; <http://dx.doi.org/10.1016/j.androl.2014.02.002>
51. Van Wyk BE, Wink M. 2018. Medicinal plants of the world. CABI, 17: 52-65
52. Akakpo C, Mensah JK, Akoto CO. Antimicrobial, antioxidant, anthelmintic and anti-inflammatory bioactivities of *Sabicea calycina*. International Journal of Biological and Chemical Sciences. 2022; 16(3): 1084-1099
53. Victor PD, Krukru EE, Okpara PE, Ajie PC, Reuben E, Amadi-Ikpa HA, Wami-Amadi CF, Otto BJ, Dan-Jumbo D, Nkpurukwe CI. The Effect of Ethanolic Extract of "*Laurus nobilis*" (Bay Leaves) on the Reproductive Characteristics of Male Wistar Rats. Scholars International Journal of Obstetrics and Gynecology. 2023; 6(4): 156-162.
54. Tietz NW (1995). Clinical Guide to Laboratory Tests. W.B. Saunders, Philadelphia, USA. 3rd edition.
55. Deinkuro N, Knapp C, Raimi M, Nanlok N. Oil Spills in the Niger Delta Region, Nigeria: Environmental Fate of Toxic Volatile Organics. 2021; 10.21203/rs.3.rs-654453/v1.
56. Althof S, Needle R. (2017). Treating Low Sexual Desire in Men. 10.1002/9781118510384.ch3.

57. Vidhya AM, Misbah AAC, Padma LL, NilofarSN, Sanaula AT. An overview: some medicinal plants as Aphrodisiac agents. *International Journal of Pharmaceutical sciences and review research*. 2022; 75(1):116-123
58. Frappier J, Toupin I, Levy J, Aubertin-Leheudre M, Karelis A. Energy Expenditure during Sexual Activity in Young Healthy Couples. *PloS one*. 2013; 8. e79342. 10.1371/journal.pone.0079342.
59. Olayinka BU, Ogungbemi RF, Abinde, OO, Lawal AR, Abdulrahaman AA, Etejere EO. Proximate and Phytochemical Compositions of Leaf and Root of (Cattle Stick) *Carpolobia lutea* G. Don. *Journal of Applied Sciences and Environmental Management*. 2019; 23 (1): 53–57
60. Rahimi R, Ghiasi S, Azimi H, Fakhari S, Abdollahi M. A review of the herbal phosphodiesterase inhibitors; Future perspective of new drugs. *Cytokine*, 2010; 49:123-129
61. Chalisa S, Prapapan T, Kornkanok I. Pattara S. Phosphodiesterase Inhibitory Activity of the Flavonoids and Xanthenes from *Anaxagorea luzonensis*. *Natural product communications*. 2015; 10(2):301-303
62. Drewes SE, George J, Khan F. Recent findings on natural products with erectile-dysfunction activity. *Phytochemistry*. 2003; 62, 1019-1025.
63. Thakur M, Dixit VK. Effect of *Chlorophytum boriviliaunum*Sant. & F. on androgenic and sexual behavior in male rats. *Indian Drugs*. 2006; 43:300–306.
64. Tajuddin SA, Abdul L, Iqbal. Aphrodisiac activity of 50% ethanolic extracts of *Myristica fragan*Houtt (nutmeg). and *Syzygiumaromaticum* (L). merr. and Pery. (Clove) in male mice: A comparative study. *BMC complementary and Alternative Medicine*. 2003; retrieved <http://www.biomedcentral.com/1472-6882/3/6>
65. Leonelli, C., Garcia, Oduvaldo, P.C. & C.M. Pereira, C.M. (2011). Copulatory efficiency and fertility in male rats exposed perinatally to flutamide, *Reproductive Toxicology*, 31 (1):10-16 <https://doi.org/10.1016/j.reprotox.2010.08.003>.
66. Ratnasooriya WD, Tayakody, JR. Effects of aqueous extract of *Alpinia Caliatatarhizones* on reproductive competence of male rats. *Acta Biological Hungarica*.2006; 57:23–35.
67. Illayperuma I, Ratnasooriya WD, Weerasooriya TR. Effect of *WithaniaSomnifera* root extract on the sexual behaviour of male rats. *Asian Journal Andrology*. 2002; 4:295–298.
68. Grande G, Barrachina F, Soler-Ventura A, Jodar M, Mancini F, Marana R, Chiloiro S, Pontecorvi A, Oliva R. Milardi D. The Role of Testosterone in Spermatogenesis: Lessons from Proteome Profiling of Human Spermatozoa in Testosterone Deficiency. *Frontiers in Endocrinology*. 2022;

13URL=<https://www.frontiersin.org/articles/10.3389/fendo.2022.852661>

DOI=10.3389/fendo.2022.852661

69. Chen L, Shib G, Huangc D, Lid Y, Mae C, Shia M, Suf B, Shig G. Male sexual dysfunction: A review of literature on its pathological mechanisms, potential risk factors, and herbal drug intervention. *Biomedicine and Pharmacotherapy*. 2019; 112:108585
70. Fugl-Meyer KS, Nilsson M, Hylander B, Lehtihet M Sexual Function and Testosterone Level in Men with Conservatively Treated Chronic Kidney Disease. *American Journal of Men's Health*. 2017; 11(4):1069-1076
71. Corona G, Maggi M. The role of testosterone in male sexual function. *Reviews in Endocrine and Metabolic Disorders*. 2022; 23. 10.1007/s11154-022-09748-3.
72. Morales A. (1996). Androgen supplementation in Practice. The treatment of erectile dysfunction associated with hypotestosteronemia. In. Oddens B.J., Vermeulen, A. (Eds). *Androgen and Aging Males*. Parthenon Publishing Group London. Pp. 233– 245.
73. Kpomah ED, Kpomah B, Arhoghro EM. Histomorphological and Biochemical Changes Induced in Male Wistar Rats by Chronic Oral Doses of *Piper guineense* Schumach. & Thonn. *Nigerian Journal of Pharmaceutical and Applied Science Research*. 2018; 7(1):44-51