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Sexual Invigorating Potentials of a Combined Extract of *Sabicea calycina* and *Carpolobia lutea* on Male Wistar Rats in Crude Oil Challenged Environment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The effect of 50, 100 and 200 mg/kg of combined ethanol extract of *S. calycina* and *C. lutea* were 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

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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. The hormonal assay showed that all doses produced a 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 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. INTRODUCTION

The South–South Geoecological 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. Abubakar et al. [1] opined that "the region is indisputably responsible for the revenue that accrues to the Nation as petroleum accounts 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 result in pollution of surface and groundwater, 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 these pollutants often herald both acute and chronic health concerns in the region which include haemotoxicity [5,6], hepatotoxicity [7], carcinogenesis through its effects on chromatin DNA [8], reduced fertility and sexual dysfunction [9-11] 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 characterized 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 from crude oil exploration and spills may even contribute toward an increased prevalent rate in the South–South geoecological zone [16,17]. "In Nigeria, the National Health Policy focuses on services like malaria, family planning, diseases like STI/HIV/AIDS, infants and maternal health exclusive of men's sexual health, although it may not be life-threatening, the fact that the man cannot fulfil 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 spouses suffer sexual dysfunction may be tempted to go outside their marriage vows to satisfy their sexual cravings and needs and this can lead to increased 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 a vital element of emotional and physical intimacy that most people crave to experience throughout their lifetime, 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 lepreurii* 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 portions" [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 consists of four phases namely excitement, plateau, orgasm and resolution". "In another classification, the psychosexual phase is divided into two interconnected activities, 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 the 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. Disorders 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 the 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, which is the persistent absence of sexual fantasy and need for sex, erectile dysfunction, 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 conditions (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), ageing and systemic disease (cardiac, hepatic, renal, pulmonary, metabolic), post organ transplant" [31-33].

Despite the medical advancements in treatment and treatment facilities for male sexual dysfunction [34,35], 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 Africa [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 [45-47] and are being explored globally as a 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 ethanol extract of the *Carpolobia lutea* and *Sabicea calycina* are two such local aphrodisiacs that are essentially used by the people of the 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.

Ethnopharmaceutically, different parts of the plant have been harnessed in the treatment and management of numerous health challenges. For example, the leaves are used as an antipyretic, remedy for ulcers, malaria, dermal infection, venereal diseases, sterility, vermifuge, taenifuge, stomach problems, diarrhea, headache, leprosy, snakebite and wounds" [49,50]. "The leaves have also been used to promote childbirth while the root bark has been implicated in 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 assist them in walking. The mashed leaves are also used to treat wounds" [52]. "The infusion is also believed to enhance memory and 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 with Bonny light crude oil (BLCO).

2. MATERIALS AND METHODS

2.1 Plant Source and Authentication

The stems of *S. calycina* and *C. lutea* were harvested in Odi town, Kolokuma Opokuma Local Government Area of Bayelsa State, Nigeria. Identification and authentication were done at the Plant Science and Biotechnology Department, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria with a voucher specimen number given as UHAE2019808 and UHAE2019809 respectively and the specimens 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*) was carefully washed with distilled water to remove sand and other impurities, the samples were then shade-dried 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℃ 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 novergicus*) 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 rooms, which were clean and well-ventilated at a temperature between 28-30^{o}C, under a 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.

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.

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 A_1 Non Crude Oil Contaminated Environment (NBLCO) + 50 mL of distilled water.

Group A_2 Non Crude Oil Contaminated Environment (NBLCO) + 50 mg/kg of composite extract

Group A_3 Non Crude Oil Contaminated Environment (NBLCO) + 100 mg/kg of compositeextract

Group A_4 Non Crude Oil Contaminated Environment (NBLCO) + 200 mg/kg of compositeextract

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 B_1 Crude Oil Contaminated Environment (BLCO) + 50 mL of distilled water.

Group B_2 Crude Oil Contaminated Environment (BLCO) + 50 mg/kg of composite extract

Group B_3 Crude Oil Contaminated Environment (BLCO) + 100 mg/kg of composite extract

Group B_4 Crude Oil Contaminated Environment (BLCO) + 200 mg/kg of composite extract

2.4.2 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 spilt homogeneously on the beddings of the metabolic cages measuring $40\,cm \times 30\,cm \times 20\,cm$ housing the animals used for the crude oil environmentally challenged groups. The animals were housed in these challenged environments for 3 weeks.

2.4.3 Female rats

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

2.5 Methods Extract Administration

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

2.6 Mating Behavioural Study

The mating behaviour 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, 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 stopwatch, and the following male sexual behaviour indices were recorded and calculated.

2.6.1 Mount Latency (ML)

This is the time interlude 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.

2.6.2 Mount Frequency (MF)

The number of times the male donned the copulatory position without achieving intromission, is often typified by raising 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).

2.6.3 Intromission Latency (IL)

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

2.6.4 Intromission Frequency (IF)

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

2.6.5 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]

2.6.6 Ejaculation Frequency (EF)

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

2.6.7 Post Ejaculatory Interval (PEI)

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

2.6.8 Copulatory Efficiency (CP)

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

2.7 Biochemical Analysis

2.7.1 Methods 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 a centrifuge (model CE-800) and the sera were collected and used for the hormonal assay.

2.7.2 Assay kits and drugs

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

2.7.3 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-Sangtic Diagnostica. 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 ± standard error of the mean. The charts were plotted using GraphPad Prism 8.

3. 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. 1A. and that of the Bonny Light Crude Oil Challenged Albino Rat are shown in Fig. 1B. The mount latencies, intromission latencies and post ejaculatory intervals showed dose-dependent decreases but the 100 and 200 mg/kg were 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 $(p < 0.05)$.

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. 2A. and that of the BLCO Challenged Albino Rat are shown in Fig. 2B. 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.

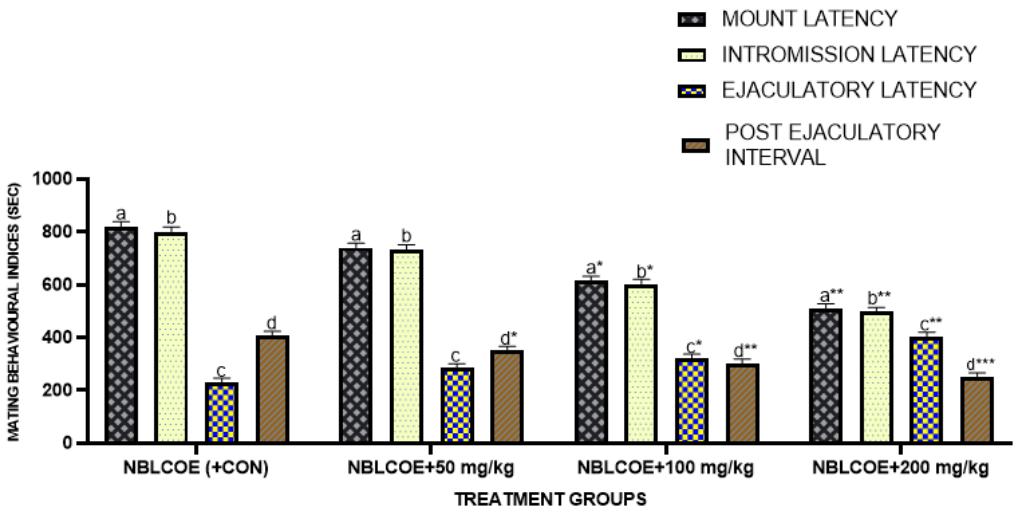

Fig. 1A. Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behavioural test (Mount latency, Intromission latency, Ejaculatory latency and Post ejaculatory interval) of NBLCO environmentally challenged albino rats

Data are Mean ± SD (n=5) mean value of parameters with the same superscript letter but with an asterisk are statistically different ($p < 0.05$), one-way Analysis of Variance (ANOVA) followed by post-hoc Tukey

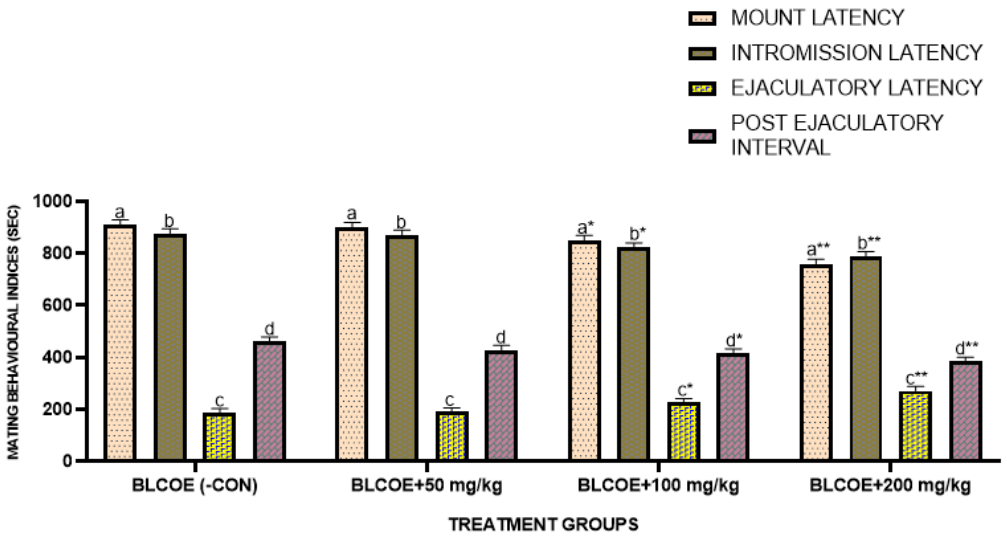

Fig. 1B. Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behavioral test (Mount latency, Intromission latency, Ejaculatory latency and Post ejaculatory latency) of BLCO environmentally challenged albino rats

Data are Mean ± SD (n=5) mean value of parameters with the 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. 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.

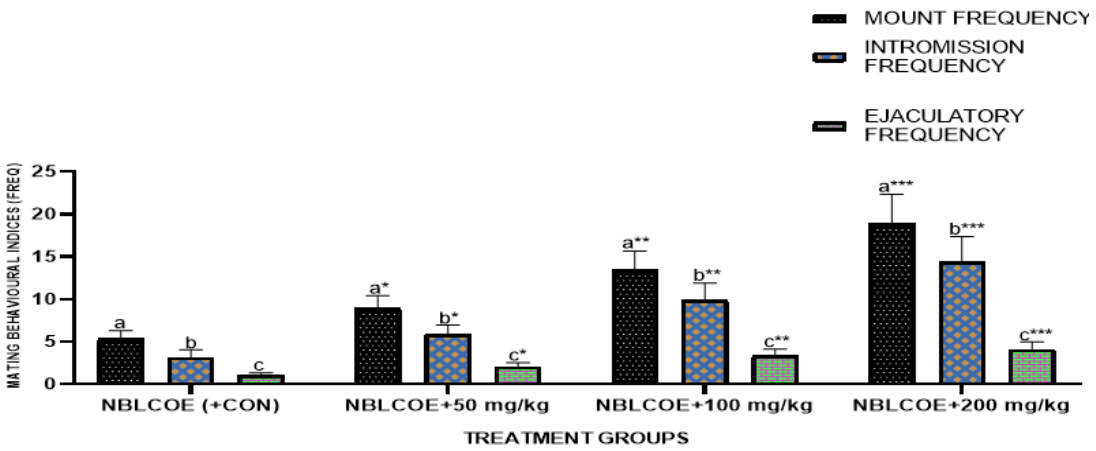

Fig. 2A. Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behavioural test (Mount frequency, Intromission frequency and Ejaculatory frequency) of NBLCO environmentally challenged albino rats

Data are Mean ± SD (n=5) mean value of parameters with the same superscript letter but with asterisks are statistically different ($p < 0.05$). *one-way Analysis of Variance (ANOVA) followed by post-hoc Tukey*

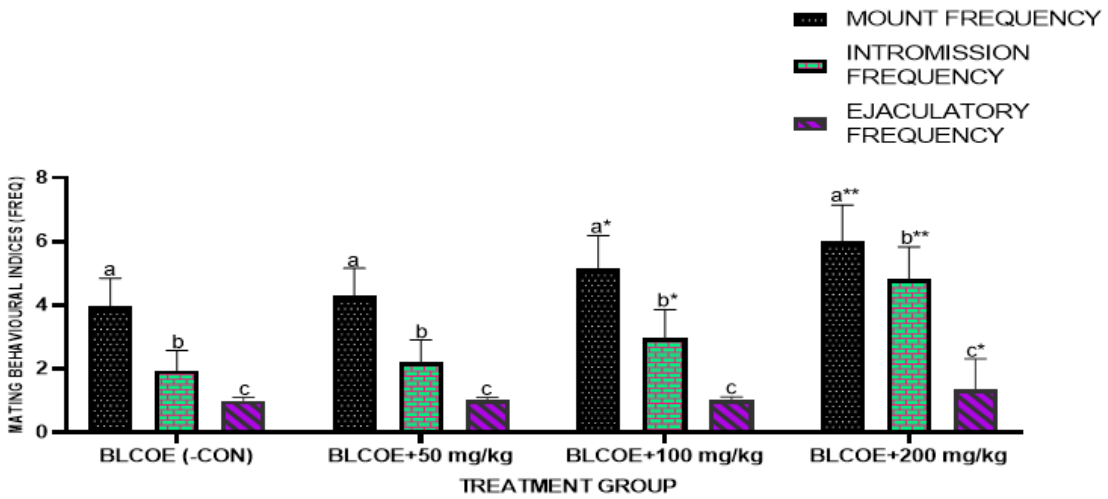

Fig. 2B. Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Mating Behavioural test (Mount frequency, Intromission frequency and Ejaculatory frequency) of BLCO environmentally challenged albino rats

Data are Mean ± SD (n=5) mean value of parameters with the same superscript letter but with asterisks 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. 4A. and that of the BLCO environmentally challenged albino rat is shown in Fig. 4B. 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.

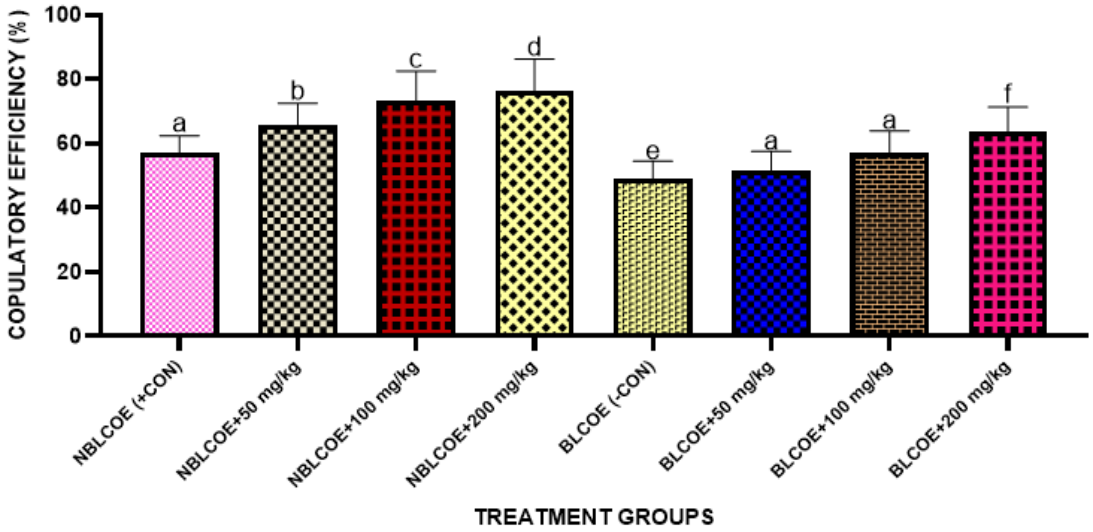

Fig. 3. Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Copulatory efficiency test of both NBLCO and BLCO environmentally challenged albino rats

Data are Mean ± SD (n=5) mean value of parameters with different superscript letters are statistically different $(p < 0.05)$, one-way Analysis of Variance (ANOVA) followed by post-hoc Tukey

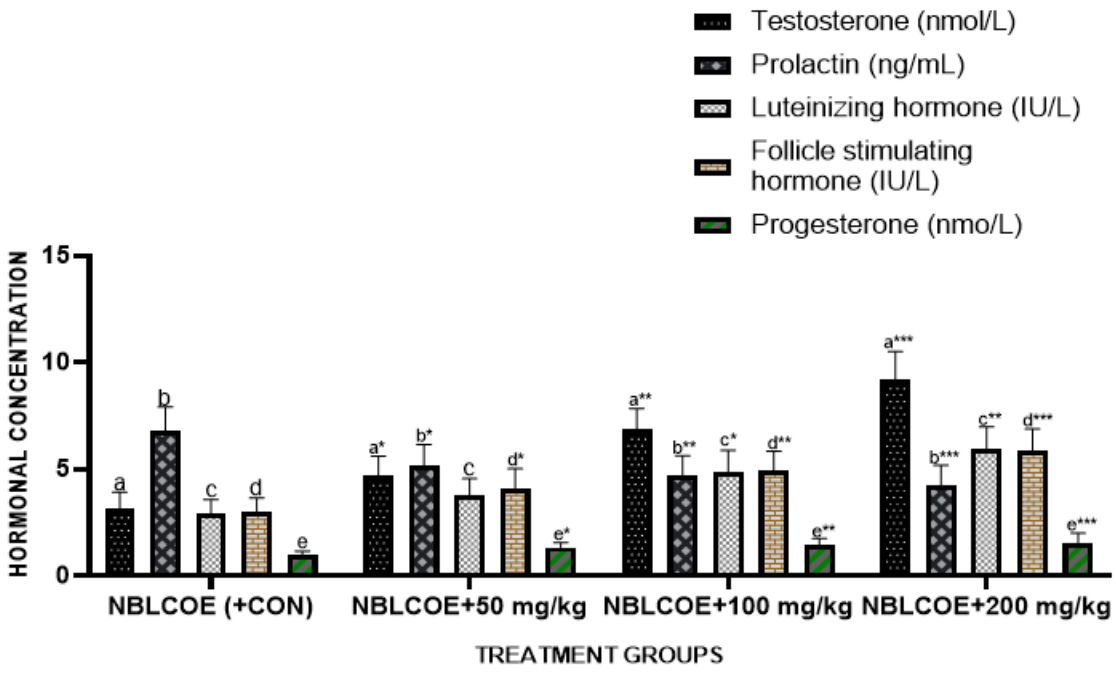

Fig. 4A. Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Reproductive Hormonal levels of NBLCO environmentally challenged albino rats

Data are Mean ± SD (n=5) mean value of parameters with the same superscript letter but with asterisks are statistically different $(p < 0.05)$. one-way Analysis of Variance (ANOVA) followed by post-hoc Tukey

4. DISCUSSION

Outside the financial remunerations accruable from crude oil, the effects of the exploration and exploitation of crude oil result in the pollution of the environment caused by spillage, causes of the spillage include but are not limited to operational errors, equipment failure and vandalism and activities of illegal refiners (sabotage). The crude oil upon contact with the environment undergoes 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 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 a 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 boosting potentials just other aphrodisiac plants that have been studied e.g. *Dactylorhiza hatagirea* and *Mondia whitel"* [18]. The significantly increased period of ejaculatory latency may be ascribed to the presence of various flavonoids in both plant extracts 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* and *Syzygium aromaticum"* [64, 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 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 *Withania somnifera* [68].

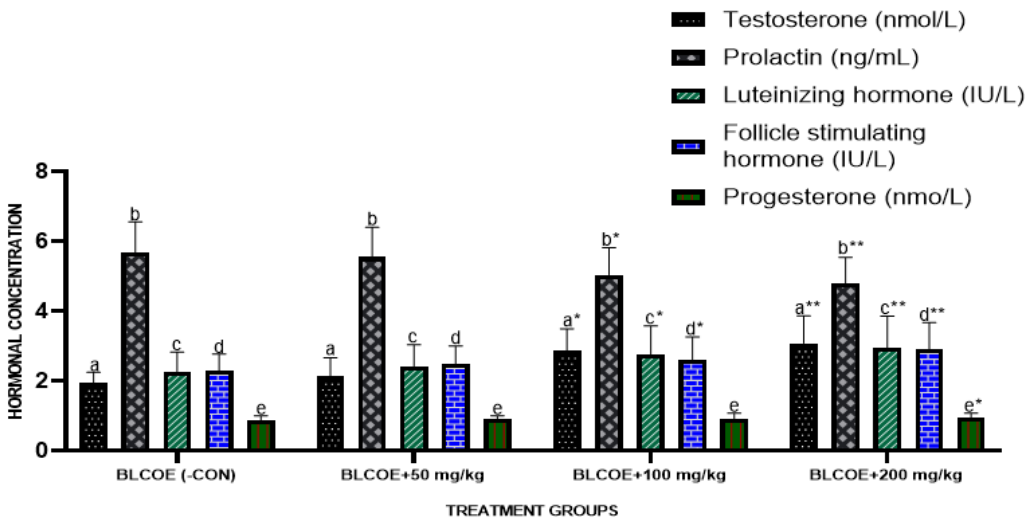

Fig. 4B. Effects of 50, 100 and 200 mg/kg of combined extract of *S. calycina* and *C. lutea* on Reproductive Hormonal levels of BLCO environmentally challenged albino rats

Data are Mean ± SD (n=5) mean value of parameters with the same superscript letter but with asterisks are statistically different ($p < 0.05$)*, one-way Analysis of Variance (ANOVA) followed by post-hoc Tukey*

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 being more effective.

4.2 Effects of 50, 100 and 200 mg/kg of a 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 in one form of sexual dysfunction or the other and a decrease in reproductive ability in men [70]. FSH is secreted by the Sertoli cells and 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 number 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 is 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 behavioural indices.

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5. CONCLUSION

Findings from this study have shown that rats challenged with BLCO had significantly reduced ($p < 0.05$) 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 heighten sexual functions in the NBLCO rats.

ETHICAL APPROVAL

Animals for the research were handled in accordance with the Institutional Animal Ethics Committee (IAEC) as per guidelines of the Committee for Control and Supervision of Experimental Animals. Protocols for the use of these animals were approved by the Directorate of Research and Quality Assurance, Federal University Otuoke, Bayelsa State vied an approval DRQA/FUO/0100/13/12/23.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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