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Research Article

Aphrodisiac properties of polyherbal formulated tea in male Wistar rats

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Keywords
Aphrodisiac, carbamazepine, polyherbal, formulation, sildenafil, testosterone.

Abstract

Polyherbal remedies are a crucial aspect of traditional medicine with combining multiple herbs in a single formulation, to enhance efficacy and reduce toxicity. This study evaluated the aphrodisiac potential of a polyherbal-formulated tea (*Zingiber officinale, Allium sativum, Azanza garckeana, Ageratum conyzoides, and Anthocleista djalonensis*). Twenty male rats were divided into five groups of four rats each. Group 1 received distilled water, groups 2 and 3 received 10 and 20 mg/kg of tea extract respectively, group 4 received 5 mg/kg of sildenafil; and group 5 received 20 mg/kg of carbamazepine orally for 14 days. The rats were assessed for mating, orientational behaviour, and testosterone. levels. The study found that polyherbal tea extract at 20 mg/kg and sildenafil (5 mg/kg) significantly increased the mounting and intromission frequency compared to the normal control and carbamazepine ($p < 0.01$). Aqueous extract of tea (10 and 20 mg/kg) and 5 mg/kg of sildenafil increased ejaculatory frequency and latency compared to normal control and carbamazepine ($p < 0.05$). Polyherbal tea (10 and 20 mg/kg), 20 mg/kg of carbamazepine, and 5 mg/kg of sildenafil significantly increased the number of anogenital sniffing, licking, and genital grooming compared to the water control ($p < 0.05$). Aqueous extract of the tea (10 and 20 mg/kg) and 5 mg/kg sildenafil increased testosterone levels, compared to the water control and carbamazepine ($p < 0.05$). The weight of the testes remained unaltered. In conclusion, polyherbal tea formulation possesses aphrodisiac properties and enhances sexual health and performance in male rats.

1. Introduction

Traditional medicine has long utilized plants and natural substances to enhance sexual health and treat sexual dysfunctions. Aphrodisiacs are integral to various traditional medical systems, employed to boost sexual desire and performance. The term originates from Aphrodite, the Greek goddess of love [1]. Polyherbal remedies, which are key components of traditional medicine, combine various herbs in a single formulation to enhance efficacy and reduce toxicity. Ancient civilizations, such as the Egyptians, Chinese, Indians, and Greeks, documented the use of medicinal plants. Traditional Chinese Medicine (TCM), for example, has a long history of using polyherbal formulations, as detailed in classic texts such as the "Huangdi Neijing" (The Yellow Emperor's Classic of Internal Medicine), which continues to influence practices today [2]. Similarly, African traditional medicine showcases a deep understanding of the natural world, using complex mixtures of medicinal plants to promote healing [3]. Traditional medicine widely recognizes ginger (*Zingiber officinale*) for its aphrodisiac properties known for its warming

effect and ability to enhance blood circulation, thereby improving sexual function and arousal. Studies have suggested that bioactive compounds, such as gingerol, stimulate blood flow and testosterone production, enhancing sexual desire [4]. Garlic (*Allium sativum*) is also acclaimed for its aphrodisiac potential in traditional medicine. Its rich allicin content enhances blood circulation, which is crucial for sexual arousal and performance. Research indicates that garlic has been shown to boost libido and testosterone levels, thereby improving sexual health [5-7]. Its antioxidant and anti-inflammatory properties help to reduce oxidative stress, enhance cardiovascular health, and support sexual vitality. Thus regular consumption of garlic is linked to improved sexual function and increased sexual desire [8]. *Azanza garckeana*, commonly known as the snot apple, is recognized in traditional African medicine for its aphrodisiac properties. The fruit is believed to enhance sexual desire and performance, which is attributed to its rich nutrient profile, including vitamins and antioxidants that improve overall vitality [9]. Studies have indicated that *Azanza garckeana* may stimulate libido and enhance sexual function by boosting energy levels and reducing oxidative stress, thereby supporting reproductive health [10]. *Ageratum conyzoides*, commonly known as goatweed, is traditionally used as an aphrodisiac in various cultures. Research suggests that *Ageratum conyzoides* improves sexual function by increasing blood flow and stimulating libido [11, 12]. Its anti-inflammatory and antioxidant properties contribute to overall reproductive health by reducing oxidative stress and improving vascular function [13]. *Anthocleista djalonensis*, a plant native to Africa, is traditionally used as an aphrodisiac. Its roots and bark are believed to enhance sexual desire and performance by stimulating libido and enhancing erectile function [14]. This study aimed to evaluate the aphrodisiac properties of a polyherbal tea formulation (*Zingiber officinale, Allium sativum, Azanza garckeana, Ageratum conyzoides,* and *Anthocleista djalonensis*).

2. Materials and methods

2.1. Plant collection

Allium sativum (garlic) and *Zingiber officinale* (ginger) were purchased from Kurmi Market in the Kano Municipal Local Government Area, Kano State. *Azanza garckeana* (Goron Tula) was purchased from Tula village in the Kaltungo Local Government Area, Gombe state. *Anthocleista djalonensis* was purchased from the Uhunmwonde Local Government Area, and *Ageratum conyzoides* (goatweed) was obtained from the Ikpoba-Okha Local Government Area in Edo State. The plant was identified and authenticated by Dr. H. Akinnibosun at the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria with the following herbarium number for *Zingiber officinale as UBH-Z384, Thespesia garckeana as UBH-A508, Anthocleista djalonensis as UBH-A594 and Ageratum conyzoides as UBH-A344.*

2.2. Preparation of plant materials

Garlic and *Zingiber officinale* (ginger) were washed and chopped into smaller pieces. The *Anthocleista djalonensis* and *Ageratum conyzoides* (goatweed) were removed from the stalk and washed thoroughly. Goron Tula seeds were collected from the fruits. The chopped garlic, ginger, and gorontula seeds were dehydrated using a dehydrator, whereas the washed *Anthocleista djalonensis* and *Ageratum conyzoides* (goatweed) were air dried. After dehydration, the dehydrated ginger, garlic, Goron Tula seeds, *Anthocleista djalonensis,* and *Ageratum conyzoides* (goatweed) were ground into powder using an industrial blender.

2.3. Formulation of polyherbal tea

The polyherbal tea was formulated according to Uwaya and Effinog [15] and Sahoo *et al*. [16] with slight modifications of *Ageratum conyzoides* (goatweed) at a ratio of 0.3. The polyherbal-formulated tea was prepared using the following formulation.

Allium sativum (garlic), *Anthocleista djalonensis*, *Zingiber officinale* (ginger), and *Ageratum conyzoides* (goatweed) at ratio of 1:1:1:1:0.3, respectively.

2.4. Polyherbal tea extraction

The formulated polyherbal tea (1161 g) was weighed into an extraction jar, and 12.25 liters of distilled water was added. The mixture was stirred using a stirrer and allowed to stand for 72 h. The mixture was filtered using a strainer into a lidded storage container. The filtrate was then concentrated using a water bath.

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The extract was stored in an amber bottle in a refrigerator at 4°C before use.

2.5. Experimental animals

Healthy adult albino male rats of either sex weighing 120 –250 g were purchased from a commercial farm in Ibadan, Oyo State. The rats were housed within the animal facility of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, and were acclimatized for two weeks, under normal laboratory conditions with a 12 h light/dark cycle. They were fed with normal animal pellets spontaneously. The animals were handled in accordance with laboratory protocols (National Institute of Health, USA, Public Health Service Policy on Humane Care and Use of Laboratory Animals, 1986) and approved by the Life Sciences Research Ethical Committee (approval number LS23110).

2.6. Experimental design

The Aphrodisiac activity was studied according to Ahmed and Aslam [17], Singh *et al*. [18], and Sahoo *et al*. [16] methods with slight modification. Twenty (20) healthy male Wistar rats were divided into 5 groups of 4 rats each as follows. Group 1: Received 2 ml/kg of distilled water. Group 2 and 3 received 10 mg/kg and 20 mg/kg aqueous extract of polyherbal-formulated tea. Group 4 received 5 mg/kg of sildenafil (positive standard drug). Group 5 received 20 mg/kg of carbamazepine (negative standard drug). The tea and drugs were administrated orally for 14 days. In preparation for mating, 20 female rats were induced into the oestrus phase artificially, with 10 µg/kg of estrogen was administered subcutaneously to the female rats 48 hprior to mating, followed by 500 µg/kg of progesterone subcutaneously 4 h before the experiment. Male rats were placed in a rectangular plexiglass chamber, followed by female rats in a ratio of 1:1. Mating behaviours such as mounting frequency (MF), intromission frequency (IF), ejaculatory frequency (EF), and ejaculatory latency, were recorded.

2.7. Blood sample collection

The animals were sacrificed using the euthanizer method by placing them in an airtight container containing chloroform (anesthetic). The animals were dissected in the abdominal region, and blood was collected via the abdominal aorta into a plain container. The testes were isolated and weighed. The blood in the plain container was centrifuged at 3000 revolutions per minute (rpm) for 10 minutes. The serum was collected and stored in a deep freezer at -20°C before analyzing for testosterone.

2.8. Procedures for analysis

2.8.1. Estimation of testosterone level

The testosterone levels were estimated using an ELISA kit (AccuBind, USA).

Procedure:

The wells of the microplates were formatted for each serum reference, control, and animal serum. 10 µL of the appropriate serum reference, control, and animal serum was pipetted into the assigned well. Furthermore, 50 µL of the ready-to-use testosterone enzyme reagent was added to all wells. To ensure proper mixing, the microplate was swirled gently for 20-30 seconds. The microplate was then covered and incubated for 60 min at room temperature. After incubation, the contents of the microplate were discarded by decantation or aspiration, and if decanted, the plate was blotted dry with absorbent paper. Next 350 µL of wash buffer was made, as detailed in the reagent preparation section, followed by decantation or aspiration. This washing step was repeated two additional times, totaling three washes, using either an automatic or manual plate washer as per the manufacturer's instructions. Subsequently, 100 µL of the working substrate solution was added to all wells, emphasizing the consistent order of reagent addition to minimize reaction time differences between wells. It was specifically noted that the plate was not shaken after this addition. It was incubated at room temperature for fifteen minutes. Finally, 50 µL of stop solution was added to each well, and the solution was gently mixed for 15-20 seconds, maintaining the same order of reagent addition to minimize time differences between wells. The absorbance of each well was measured at 450 nm using a reference wavelength of 620-630 nm to minimize well imperfections in a microplate reader (model: Auto Elisa P). The results were read within 30 min of adding the stop solution.

2.8.2. Statistical analysis

Data were presented as mean ± standard error of the mean (SEM), with 'n indicating the number of rats in

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each experimental group. One-way analysis of variance (ANOVA) was conducted, followed by the Newman-Keuls post hoc test. GraphPad Prism software version 9 was used for all data analyses. A significance level of $P < 0.05$ indicated notable differences between the data.

3. Results

The results shown in Fig. 1 indicate the effect of polyherbal-formulated tea on mount frequency. 5 mg/kg of sildenafil citrate and 20 mg/kg of aqueous extract of the polyherbal tea increase the mount frequency compared to the water control and carbamazepine ($p < 0.01$). Fig. 2 showed the effect of polyherbal-formulated tea on intromission frequency. Sildenafil citrate (5 mg/kg) and 20 mg/kg of aqueous extracts of the polyherbal tea increased the intromission frequency when compared to water control and carbamazepine ($p < 0.01$; $p < 0.05$). Fig. 3 showed the effect of polyherbal-formulated tea on ejaculatory frequency. sildenafil citrate (5 mg/kg), 10 and 20 mg/kg of aqueous extracts of the polyherbal tea increased the ejaculatory frequency when compared to water control and carbamazepine ($p < 0.01$; $p < 0.05$). Fig. 4 showed the effect of polyherbal-formulated tea on ejaculatory latency. Sildenafil citrate (5 mg/kg), 10 mg/kg and 20 mg/kg of aqueous extracts of the polyherbal tea increased ejaculatory latency when compared to the water control and carbamazepine ($p < 0.01$; $p < 0.05$). Fig. 5 showed the effect of polyherbal formulated tea on anogenital sniffing. The administration of 20 mg/kg of carbamazepine, 5 mg/kg of sildenafil citrate, and 20 mg/kg of aqueous extract of the polyherbal tea increased anogenital sniffing when compared to the water control (**$p < 0.01$; *$p < 0.05$). Fig. 6 showed the effect of polyherbal-formulated tea on licking. Compared to water control, 20 mg/kg of carbamazepine, 5 mg/kg of sildenafil citrate, and 20 mg/kg of aqueous extract of the polyherbal tea increased licking (**$p < 0.01$; *$p < 0.05$). Fig. 7 showed the effect of polyherbal-formulated tea on genital grooming. 20 mg/kg of carbamazepine, 5 mg/kg of sildenafil citrate, and 20 mg/kg of aqueous extract of the polyherbal tea increased genital grooming when compared to the water control (**$p < 0.01$; *$p < 0.05$). Fig. 8 showed the effect of polyherbal-formulated tea on testosterone levels. Compared to the water control and carbamazepine groups, sildenafil citrate (5 mg/kg) and aqueous extracts (10 and 20 mg/kg) of polyherbal tea increased testosterone levels, (**$p < 0.01$; *$p < 0.05$). Fig. 9 shows no effect of polyherbal formulated tea on testis body weight ratio($p>0.05$).

4. Discussion

Globally, plants have been embraced by various cultures as a safe and natural form of medicine. Many botanical products have demonstrated the potential for increasing fertility in both genders, highlighting the urgent need to investigate further natural plant sources to tackle the rising issues of sexual dysfunction worldwide. This focus is underpinned by their vital role in promoting human health, particularly in reproductive wellness [19]. Various herbs, when used in combination (polyherbal), are employed to treat conditions such as sexual dysfunction and stimulate sexual arousal [20]. For these plant or herbal combinations, they must undergo rigorous testing for their biological efficacy to gain scientific acceptance. The commonly used scientific tests to assess sexual activity in plants include mount behaviour, mating performance, libido, orientation behaviour, and hormonal analysis [21]. The criteria of sexual behaviour that are commonly observed in research include latency and frequency of mounts, intromission frequency and latency, ejaculatory frequency, and latency, as well as behaviors like anogenital sniffing, licking, and genital grooming [17]. This study evaluated the effects of the aqueous extract from a polyherbal tea on male sexual competence in rats, comparing it with sildenafil as a positive control and using carbamazepine and water as negative controls. The frequencies of mount (MF) and intromission (IF) are important indices of sexual vigor, libido, and potency. Specifically, the mount frequency (MF) indicates sexual motivation, and an increase in intromission frequency (IF) reflects the effectiveness of erections, penile orientation, and activation of ejaculatory reflexes. The administration of the aqueous extract from the polyherbal tea at 20 mg/kg increased mount frequency (MF) and intromission frequency (IF), indicating enhanced sexual vigor/libido compared to the groups receiving only water or carbamazepine (Figs. 1 and 2). Fouche

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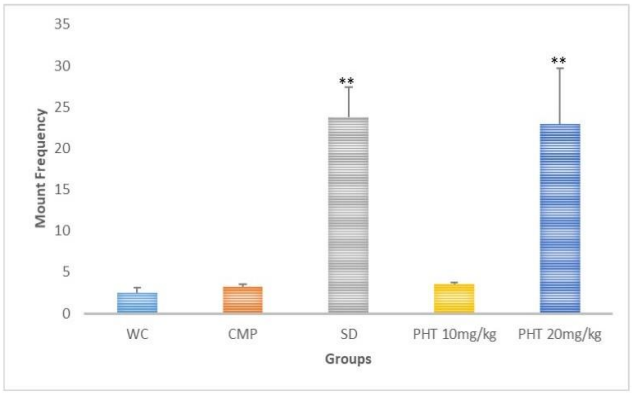

Figure 1. Effects of polyherbal tea (*Zingiberofficinale, Allium sativum, Azanzagarckeana, Ageratum conyzoides, and Anthocleistadjalonensis)* on mount frequency in male rats. 5 mg/kg of sildenafil citrate and 20 mg/kg of aqueous extract of the polyherbal tea significantly increase mount frequency when compared to water control and carbamazepine (p <0.01). WC: Water Control, CMP: Carbamazepine, SD: Sildenafil citrate, PHT: Polyherbal tea. The values are represented as ± S.E.M., n = 4.

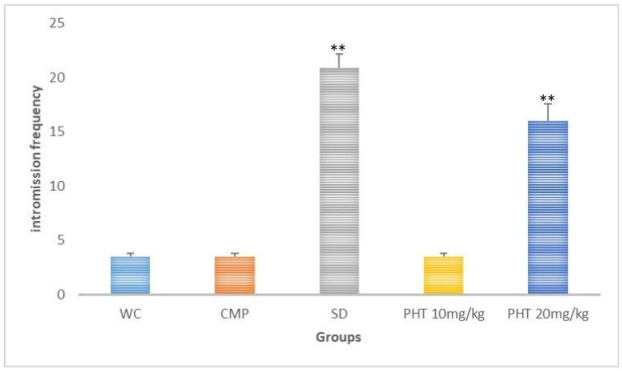

Figure 2. Effects of polyherbal tea (*Zingiberofficinale, Allium sativum, Azanzagarckeana, Ageratum conyzoides, and Anthocleistadjalonensis)* on intromission frequency in male rats. 5 mg/kg of sildenafil citrate and 20 mg/kg of aqueous extract of the polyherbal tea significantly increase intromission frequency when compared to water control and carbamazepine (**p <0.01; *p <0.05). WC: Water Control, CMP: Carbamazepine, SD: Sildenafil citrate, PHT: Polyherbal tea. The values are represented as ± S.E.M., n = 4.

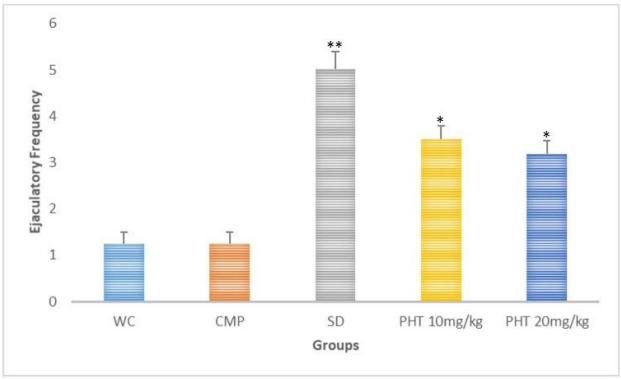

Figure 3. Effects of polyherbal tea (*Zingiberofficinale, Allium sativum, Azanzagarckeana, Ageratum conyzoides, and Anthocleistadjalonensis)* on ejaculatory frequency in male rats. 5 mg/kg of sildenafil citrate, 10 and 20 mg/kg of aqueous extract of the polyherbal tea significantly increased ejaculatory frequency when compared to the water control and carbamazepine (**p <0.01; *p <0.05). WC: Water Control, CMP: Carbamazepine, SD: Sildenafil citrate, PHT: Polyherbal tea. The values are represented as ± S.E.M., n = 4.

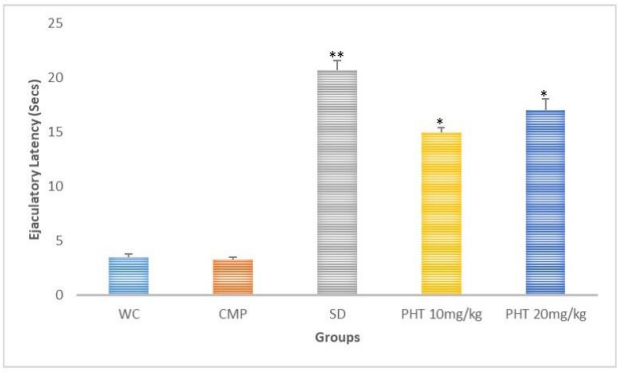

Figure 4. Effects of polyherbal tea (*Zingiberofficinale, Allium sativum, Azanzagarckeana, Ageratum conyzoides, and Anthocleistadjalonensis)* on ejaculatory latency in male rats. 5 mg/kg of sildenafil citrate, 10 and 20 mg/kg of aqueous extract of the polyherbal tea citrate significantly increased ejaculatory latency when compared to the water control and carbamazepine (**p <0.01; *p <0.05). WC: Water Control, CMP: Carbamazepine, SD: Sildenafil citrate, PHT: Polyherbal tea. The values are represented as ± S.E.M., n = 4.

et al. [9], observed similar effects with the aqueous extract of aerial parts of *Monsonia angustifolia* in male Wistar rats. The administration of the aqueous extract from the polyherbal-formulated tea at doses of 10 and 20 mg/kg significantly increased ejaculation frequency and latency, suggesting that the polyherbal tea might prolong the copulation period (Figs. 3 and 4). These results indicate improved sexual vigor, potency, motivation, and copulatory performance in the treated rats, highlighting the beneficial effects of polyherbal tea's on key indicators of sexual health and performance. Furthermore, the increase in ejaculatory latency is a strong indicator of enhanced sexual function, specifically a prolonged duration of coitus, which implies aphrodisiac activity. Similar findings were reported by Erhabor and Idu [22], supporting the efficacy of polyherbal tea in enhancing sexual function. The administration of polyherbal tea at 20

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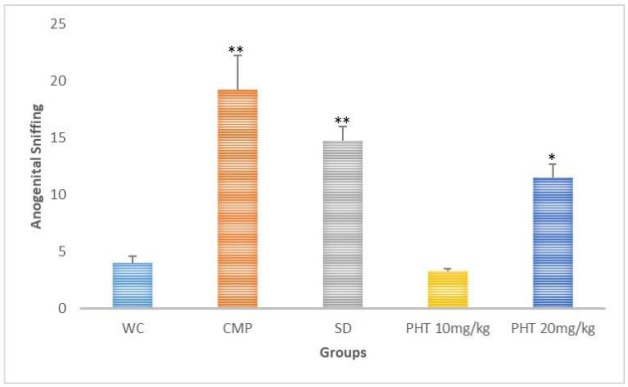

Figure 5. Effects of polyherbal tea (*Zingiberofficinale, Allium sativum, Azanzagarckeana, Ageratum conyzoides, and Anthocleistadjalonensis)* on anogenital sniffing in male rats. 20 mg/kg of carbamazepine, 5 mg/kg of sildenafil citrate, and 20 mg/kg of aqueous extract of the polyherbal tea significantly increased anogenital sniffing when compared to the water control and 10 mg/kg of aqueous extract (**p <0.01; *p <0.05). WC: Water Control, CMP: Carbamazepine, SD: Sildenafil citrate, PHT: Polyherbal tea. The values are represented as ± S.E.M., n = 4.

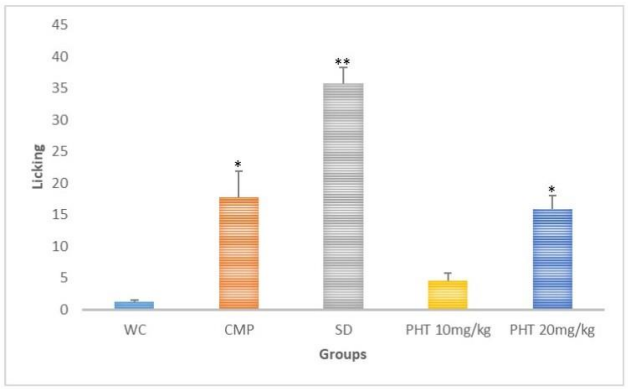

Figure 6. Effects of polyherbal tea (*Zingiberofficinale, Allium sativum, Azanzagarckeana, Ageratum conyzoides, and Anthocleistadjalonensis)* on licking in male rats. 20 mg/kg of carbamazepine, 5 mg/kg of sildenafil citrate, and 20 mg/kg of aqueous extract of the polyherbal tea significantly increased anogenital sniffing when compared to the water control and 10 mg/kg of aqueous extract (**p <0.01; *p <0.05). WC: Water Control, CMP: Carbamazepine, SD: Sildenafil citrate, PHT: Polyherbal tea. The values are represented as ± S.E.M., n = 4.

mg/kg, in conjunction with 20 mg/kg of carbamazepine and 5 mg/kg of sildenafil, significantly increased the number of anogenital sniffing, licking, and genital grooming compared to the water control (Figs. 5-7). These orientational behaviours, which are frequently linked to sexual attraction, help different

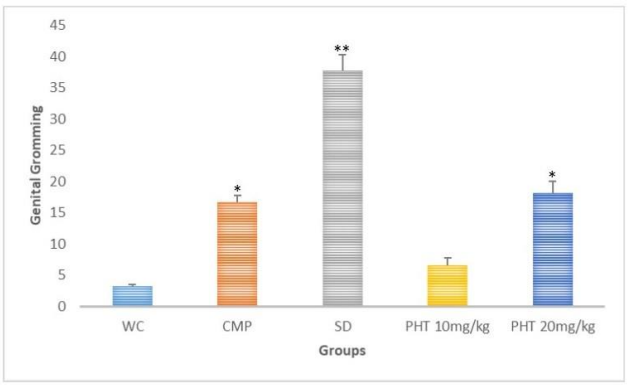

Figure 7. Effects of polyherbal tea (*Zingiberofficinale, Allium sativum, Azanzagarckeana, Ageratum conyzoides, and Anthocleistadjalonensis)* on genital grooming in male rats. 20 mg/kg of carbamazepine, 5 mg/kg of sildenafil citrate, and 20 mg/kg of aqueous extract of the polyherbal tea significantly increased genital grooming when compared to the water control and 10 mg/kg of aqueous extract (**p <0.01; *p <0.05). WC: Water Control, CMP: Carbamazepine, SD: Sildenafil citrate, PHT: Polyherbal tea. The values are represented as ± S.E.M., n = 4.

mammalian species, including rats, establish sexual hierarchies and readiness. The observed increase in these behaviours indicates an elevated state of sexual arousal, which may facilitate the commencement of sexual activities. Testosterone is the primary male hormone produced by the Leydig cells in the testes. In these cells, luteinizing hormone (LH), a type of gonadotropin, binds to receptors and stimulates the production and release of testosterone [23]. A significant increase in testosterone levels was observed following the administration of 10, and 20 mg/kg of the polyherbal tea, suggesting that the extract might mimic LH's role in activating Leydig cells (Fig. 8). These findings are consistent with previous research by JianFeng *et al.* [23], which also linked with elevated testosterone levels with an increase in sexual desire and libido. Sildenafil citrate is a potent and selective PDE-5 inhibitor and was the first drug of this type approved for erectile dysfunction treatment. This drug works by actively inhibiting the PDE-5 enzyme, which increases cyclic guanosine monophosphate (cGMP) levels and promotes relaxation of the smooth muscle in the penis. The use of PDE5 inhibitors for erectile dysfunction may involve both peripheral and central mechanisms. Peripherally, this drug may modulate

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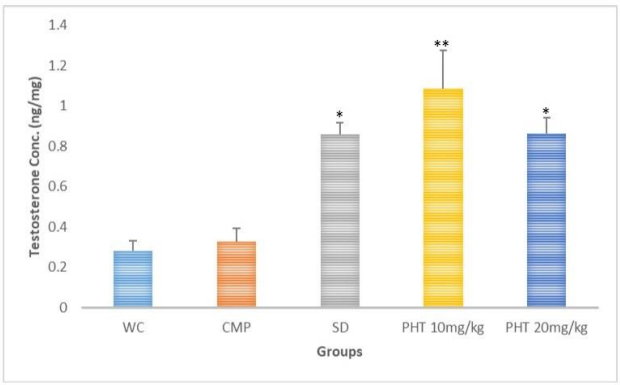

Figure 8. Effects of polyherbal tea (*Zingiberofficinale, Allium sativum, Azanzagarckeana, Ageratum conyzoides, and Anthocleistadjalonensis)* on testosterone levels in male rats. 5 mg/kg of sildenafil citrate, 10 and 20 mg/kg of aqueous extract of the polyherbal tea significantly increased testosterone levels when compared to the water control and carbamazepine (**p <0.01; *p <0.05). WC: Water Control, CMP: carbamazepine SD: Sildenafil citrate, PHT: Polyherbal tea. The values are represented as ± S.E.M., n=4.

the contractile response of the vas deferens, seminal vesicles, prostate, and urethra; induce peripheral analgesia; and extend the duration of an erection. Centrally, they might reduce sympathetic nervous system output [24]. The polyherbal formulated tea has demonstrated aphrodisiac properties, suggesting their action could be similar to that of sildenafil. The testis body weight ratio did not significantly change, as seen in Fig. 9. The testis to body weight ratio is an indicator commonly used to evaluate the overall health and functionality of the reproductive organs. The absence of notable effects on this parameter indicates that although polyherbal tea increases sexual behaviour, it does not negatively impact the physiological health of the reproductive organs at the administered doses. In this study, carbamazepine was used as the standard negative control. The drug is known to inhibit the production of testosterone in the gonads and influences other sex steroid hormones by activating liver enzymes. Carbamazepine is associated with a range of sexual dysfunctions, which are notably more common among individuals with epilepsy than in the general population. Men who use carbamazepine may experience a reduction in testosterone levels, which can lead to decreased libido, erectile dysfunction, and other sexual problems. Furthermore, sexual dysfunction is a prominent adverse effect of carbamazepine, which may cause diminished sexual desire, difficulty in achieving orgasm, and challenges in maintaining erections [25].

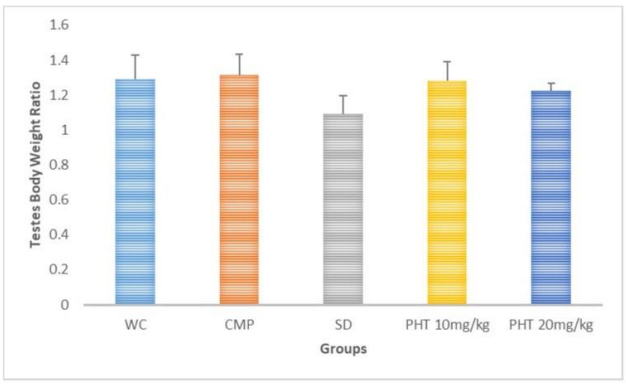

Figure 9. Effects of polyherbal tea (*Zingiberofficinale, Allium sativum, Azanzagarckeana, Ageratum conyzoides, and Anthocleistadjalonensis)* on testis body weight ratio. The polyherbal tea did not affect the testicular weight (p> 0.05). WC: Water Control, CMP: Carbamazepine, SD: Sildenafil citrate, PHT: Polyherbal tea. The values are represented as ± S.E.M., n = 4.

5. Conclusions

This study provides compelling evidence for the efficacy of polyherbal teas in enhancing sexual health and performance in male rats. However, the safety profile of this herbal combination needs to be ascertained.

Ethical statement

The animals were handled in accordance with laboratory protocols (National Institute of Health, USA, Public Health Service Policy on Humane Care and Use of Laboratory Animals, 1986) and approved by the Life Sciences Research Ethical Committee (approval number LS23110).

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, Copilot, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conceptualization, D.O.U.; methodology, D.O.U., S.N.M.; validation, D.O.U., S.N.M., J.C.A.; formal

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analysis, S.N.M., J.C.A.; investigation, S.N.M., J.C.A., resources, D.O.U., S.N.M., J.C.A.; data curation, S.N.M., J.C.A.; writing–original draft presentation, S.N.M.; writing –review and editing, D.O.U.; visualization, D.O.U., S.N.M., J.C.A.; supervision, D.O.U.

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Availability of data and materials

All relevant data are within the paper and its supporting information files. Additional data will be made available on request according to the journal policy.

Conflicts of interest

There is no conflict of interest, the authors claim.

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