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Effect of Polyherbal Formulated Teaonsperm Count, Motility and Viability in Wistar Rats

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Abstract

Polyherbal formulations have attracted increasing scientific interest because their combined constituents may produce synergistic therapeutic effects. This study evaluated the effects of an aqueous polyherbal tea extract on sperm count, motility, viability, and testicular oxidative stress markers in male Wistar rats. Twenty rats were randomly allocated into five groups of four animals: a distilled-water control group, a carbamazepine-treated group receiving 20 mg/kg, a sildenafil citrate-treated group receiving 5 mg/kg, and two treatment groups receiving 5 or 10 mg/kg of the polyherbal tea extract orally for 21 days. Following treatment, the animals were sacrificed by cervical dislocation, and sperm count, progressive and non-progressive motility, and viability were assessed. Testicular tissue homogenates were also analysed for superoxide dismutase, catalase, glutathione, and malondialdehyde. The polyherbal tea produced no significant change in sperm count compared with the distilled-water and carbamazepine groups ($p > .05$). However, the extract significantly increased progressive sperm motility and sperm viability while reducing immotile sperm, non-progressive motility, and non-viable sperm ($p < .05$ or $p < .01$). Superoxide dismutase and catalase activities were significantly elevated following treatment with the polyherbal tea compared with the distilled-water and carbamazepine groups ($p < .05$). The extract and sildenafil citrate also significantly reduced malondialdehyde levels ($p < .05$), whereas glutathione levels remained unchanged across the treatment groups ($p > .05$). These findings demonstrate that the aqueous polyherbal tea extract improves sperm motility and viability without affecting sperm count, potentially through enhanced testicular antioxidant activity and reduced lipid peroxidation. The study provides preliminary evidence supporting further investigation of the formulation as a potential intervention for improving male reproductive function.

Keywords: Antioxidant Activity; Male Reproductive Function; Polyherbal Tea; Sperm Motility; Sperm Viability

INTRODUCTION

Natural resources and their products are the most common sources of bioactive molecules in medicines since the development of other advanced treatments after thegenomic era (Kutama *et al.,* 2018). The World Health Organisation (WHO) describes traditional herbal medications as naturally occurring, plant-derived materials with negligible or no industrial processing which have been utilised to treat ailments within regional or local healing practices (Tilburt and Kaptchuk, 2008). Traditional systems of medicine and their formulations have been extensively used for many years in developed and developing countries, mainly due to their natural origin and minor side effects (Balunas and Kinghorn, 2005). Herbal medicine is in the mainstream of around 75–80% of the world population, primarily in the developing countries, for use in primary health care to treat diseases (Qazi and Molvi, 2016). This is mostly because of the universal belief that herbal medicines don't have any side effects, in addition to being locally available and cheap (Qazi and Molvi, 2016). The treatment of injury or disease by plants or plant material, either in the crude or processed state, is known as traditionall herbal medicine (Sadiku *et al.,* 2022). Plants have been used in a single or combined form in the treatment of diseases (Msomi and Simelane, 2017). Herb-herb combinations, also known as polyherbal therapy, have been used in Chinese medicine practice for thousands of years (Che *et al.,* 2013). Drug combination often produces a promising effect in the treatment of diseases over a single drug. The concept of drug combination has been well established in Western medicine, and remarkable success has been achieved over the decades. Though this progress is made, scientific evidence of therapeutic benefits is still lacking in herbal medicine (Balkrishna *et al.,* 2024).

Plant Composition

Plants used include *Curcuma longa, Anthocleistadjaalonesis, Mucuna puriens, Musa paradisiaca and Thespesia garckeana.*

Plant Collection

Curcuma longa (turmeric) was purchased from Kurmi Market within Kano Municipal Local Government Area, Kano State. *Thespesia garckeana* (Gorontula) was purchased from Gombe State. *Anthocleistadjalonensis* and *Musa paradisiaca* (banana root) were gotten from Ikpoba-Okha Local Government Area in Edo State. *Mucuna pruriens* (velvet beans) was purchased from Igbanke in Orhionmwon.Local Government Area, Edo State. The plants were identified and authenticated by Dr H. Akinnibosun in the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Edo State.

Preparation of Plant Materials

Thespesia garckeana (Goron Tula) seeds were collected from the fruit. The *Curcuma longa* (turmeric) was washed in running water and chopped into smaller bits. The*sis and Mucuna pruriens (Velvet* Beans) were removed from the stalk and also washed in running water. The *Musa paradisiaca* (banana root) was air-dried and broken into smaller bits. The chopped *Curcuma longa* and *Thespesia garckeana* seeds were dehydrated using a dehydrator at 50°C, while the washed *Anthocleistadjalonensis* and *Mucuna pruriens* were air-dried. After dehydration, the dehydrated *Thespesia garckea*, *Curcuma longa*, *Mucuna pruriens*, *Musa paradisiaca* and *Anthocleistadjalonensis* were ground to powder separately using an industrial blender (KENWOOD MODEL; KCB239k). The plants were weighed separately using an analytical balance and mixed together.

Formulation of Polyherbal Tea

The polyherbal formulated tea was formulated according to Uwaya and Effiong (2024). with slight modification. The polyherbal tea was prepared using the following formulation of *Musa paradisiaca, Curcuma longa, Anthocleistadjalonensis, Mucuna pruriens and Thespesia garckeana* in the ratio of 1:1:1:0.1:0.5, respectively.

Polyherbal Tea Extraction

Formulated polyherbal tea (648 grams) was weighed into an extracting jar, and 9.75

litres of distilled water were added. The mixture was stirred using a stirrer and allowed to stand for 72 hours. The mixture was filtered using a strainer into a storage container with a lid. The filtrate was concentrated over a water bath. The extract was stored in a storage bottle in a refrigerator at 4°C prior to use.

Experimental Design

The sperm count, motility, and viability were studied according to the method of Naghdi *et al.,* 2016, with slight modification. Twenty healthy male albino rats were allotted into five groups of four rats in each group as follows:

Group 1 – Received 2 ml/kg of distilled water.

Group 2 – Received 20 mg/kg of carbamazepine (standard drug).

Group 3 received 5 mg/kg of sildenafil (standard drug).

Group 4 – Received 10 mg/kg aqueous extract of polyherbal-formulated tea.

Group 5 – Received 20 mg/kg aqueous extract of polyherbal-formulated tea.

The control group, standard group, and extracts group were administered orally for 21 days.

Epididymal Sperm Preparation

After 21 days, the animals were sacrificed using chloroform as anaesthesia, after which the testes, epididymis, and seminal vesicles were removed, isolated, and transferred into a Petri dish containing 1 ml of phosphate buffer after ensuring the adherent tissue was cleared from the cordial epididymis. The caudal epididymis was cut open to release the sperm in it and was left for 15-30 minutes.

Sperm Count Assessment

The sperm count was evaluated by diluting one ml of sperm suspension with one ml of sperm dilution fluid. Ten microlitres of the mixture were transferred into a hemocytometer, and the sperm count was assessed per 250 tiny squares of the hemocytometer (Naghdi *et al.,* 2016).

Sperm Motility Assessment

Two drops of the sperm were dropped on a clean slide with the aid of a Pasteur pipette. The slides were covered with a cover slip and viewed under a light microscope at x40 magnification. The progressive motility, nonprogressive motility and immotile were observed, counted and recorded (Naghdi *et al.,* 2016).

Sperm Viability Assessment

To determine the percentage of viable sperm, 7 μL of trypan blue was mixed with 20 μL of sperm suspension. The percentage of live (unstained) and dead (blue stained) spermatozoa was measured using a light microscope at x400 magnification (Naghdi *et al.,* 2016).

Histological Assessment

The tissue specimen was dehydrated in alcohol, processed in xylene and then embedded in paraffin wax before sectioning with a microtome. Sections (5µm thick) of the tissues were stained with haematoxylin and eosin. All sections were viewed under a Leica light microscope (Model DM500).

RESULTS

The effect of polyherbal formulated tea (*Mucuna pruriens*, *Curcuma longa, Anthocleista djanalonsis, Musa paradisaca and Thespesia garckeana)* on sperm count, viability and motility

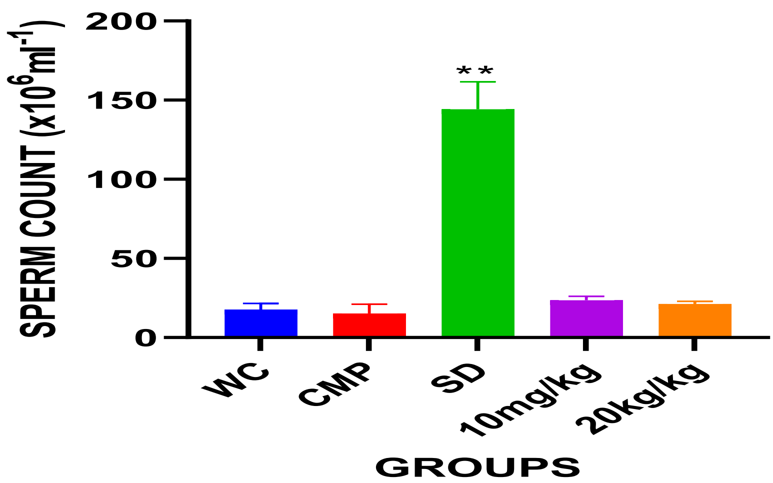


Figure 1: The effect of polyherbal formulated tea (*Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca and Thespesia garckeana*) on sperm count,

The polyherbal formulated tea at 10 and 20 mg/kg has no effect on the sperm count when compared with normal control and Carbamazepine ($p > 0.05$). While Sildenafil Citrate showed a significant increase in sperm count when compared with normal, Carbamazepine and the polyherbal formulated tea at 10 and 20 mg/kg (**p < 0.01). Values were presented as mean ± S.E.M. n=4

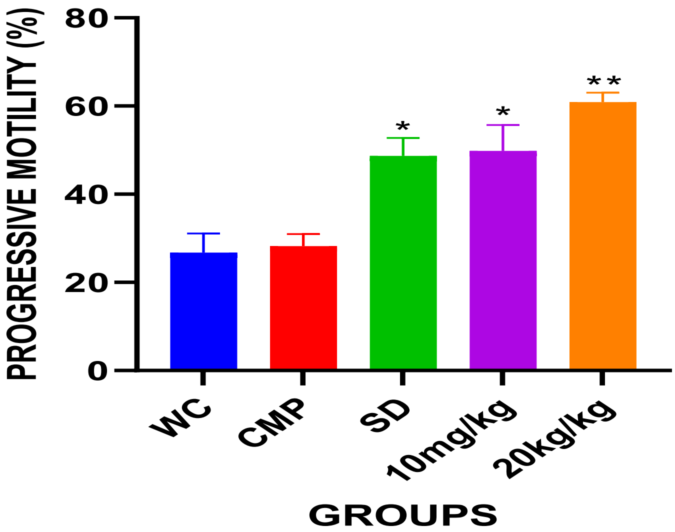


Figure 2: The effect of polyherbal formulated tea (*Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca and Thespesia garckeana*) on Sperm Progressive Motility.

The aqueous extract of polyherbal formulated tea at 10 and 20 mg/kg and Sildenafil Citrate significantly increased sperm progressive motility when compared with water control and Carbamazepine (**p<0.01; *p<0.05). Values were presented as mean ± S.E.M. n=4

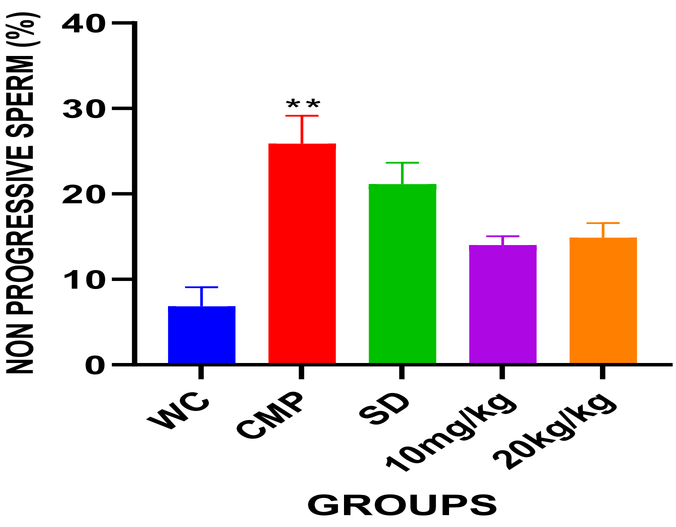


Figure 3: The effect of polyherbal formulated tea tea (*Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca and Thespesia garckeana*) on Non Progressive Sperm Motility.

The aqueous extract of polyherbal formulated tea at 10 and 20 mg/kg and sildenafil citrate significantly reduced non-progressive sperm motility when compared to carbamazepine (**$p < 0.01$). Values were presented as mean ± S.E.M. n=4

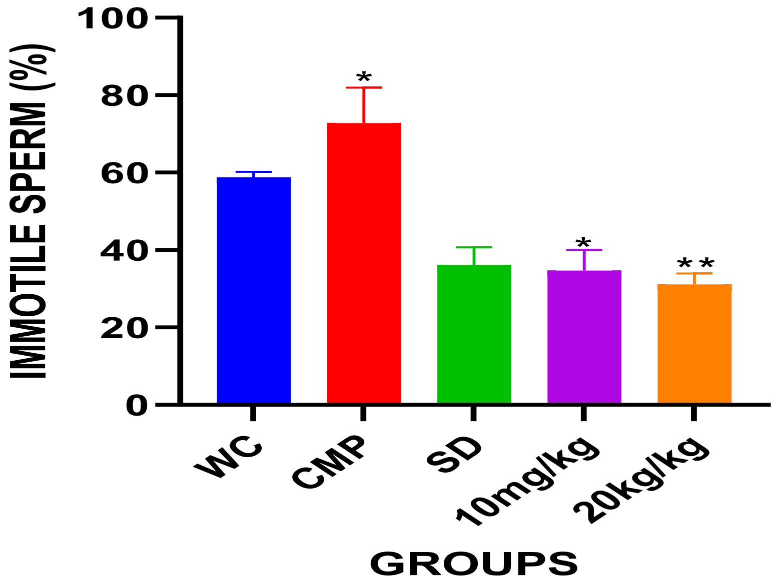


Figure 4: The effect of polyherbal formulated tea tea (*Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca and Thespesia garckeana*) on immotile sperm.

The aqueous extract of polyherbal formulated tea at 10 and 20 mg/kg significantly reduced immotile sperm when compared with water control and carbamazepine (**$p < 0.01$). Values were presented as mean ± S.E.M. n=4

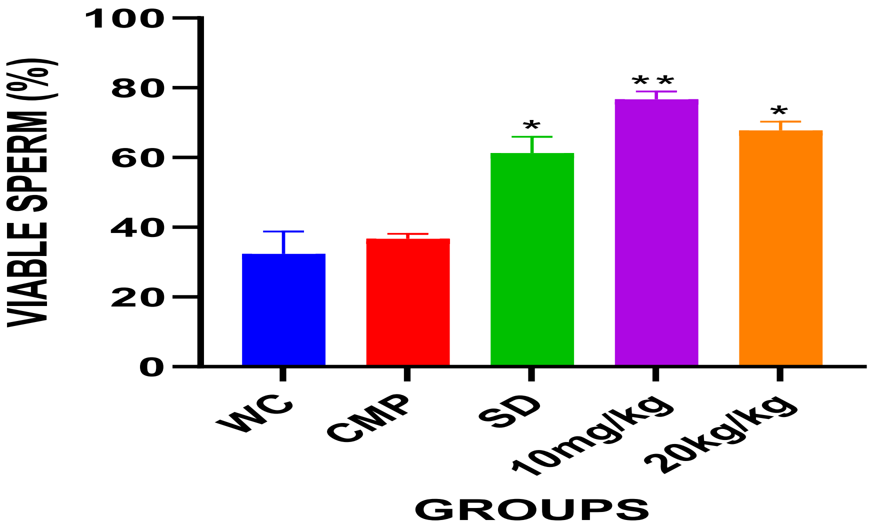


Figure 5: The effect of polyherbal formulated tea tea (*Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca and Thespesia garckeana*) on Viable Sperm.

The aqueous extract of polyherbal formulated tea at 10 and 20 mg/kg and Sildenafil Citrate significantly increased the viable sperm when compared with water control and Carbamazepine (**$p<0.01$; *$p<0.05$). Values were presented as the mean ± S.E.M. n = 4.

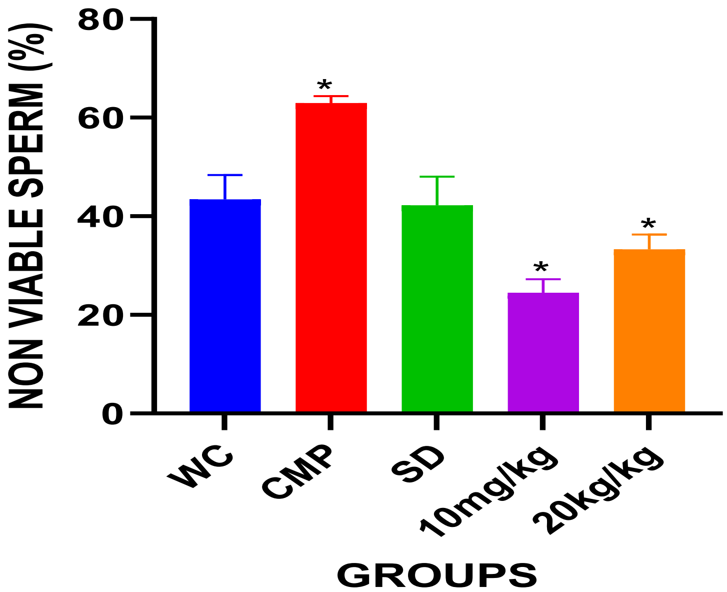


Figure 6: The effect of polyherbal formulated tea tea (*Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca and Thespesia garckeana*) on Non Viable

Sperm.

The aqueous extract of polyherbal formulated tea at 10 and 20 mg/kg significantly reduced the non-viable sperm when compared with carbamazepine and normal control (*$p<0.05$; *$p<0.05$). Values were presented as mean ± S.E.M. n=4

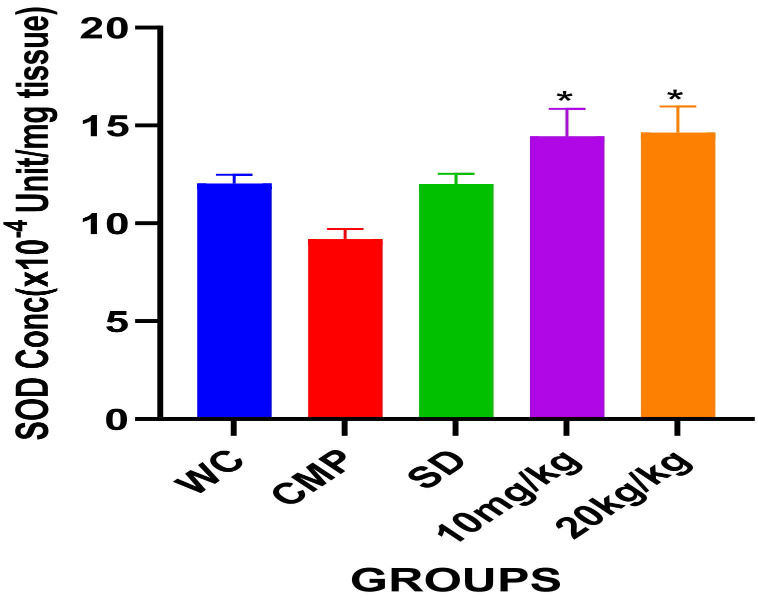


Figure 7: The effect of polyherbal formulated tea tea (*Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca and Thespesia garckeana*) on Superoxide dismutase (SOD).

The aqueous extract of polyherbal formulated tea at 10 and 20 mg/kg significantly increased the superoxide dismutase (SOD) when compared with the normal control (*$p<0.05$). Values were presented as the mean ± S.E.M. n = 4.

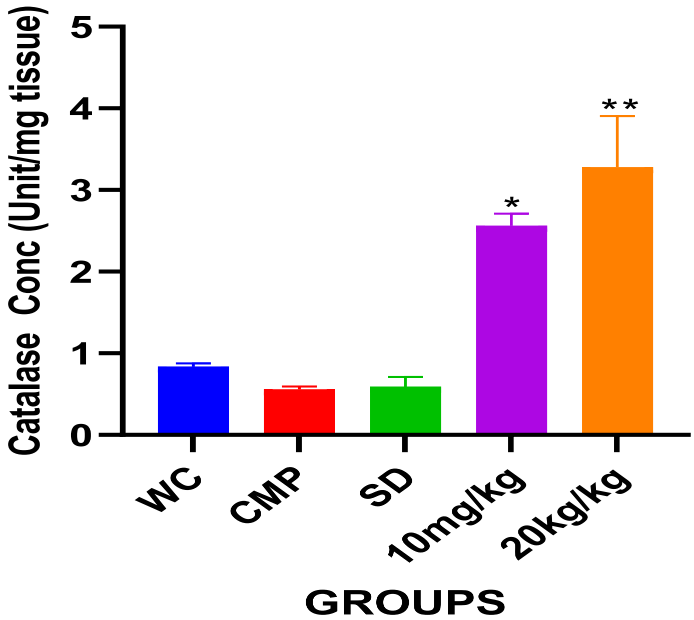


Figure 8: The effect of polyherbal formulated tea tea (*Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca and Thespesia garckeana*) on Catalase (CAT).

The aqueous extract of polyherbal formulated tea at 10 and 20 mg/kg significantly increased the Catalase (CAT) when compared with water control. (**p<0.01). Values were presented as mean ± S.E.M. n=4.

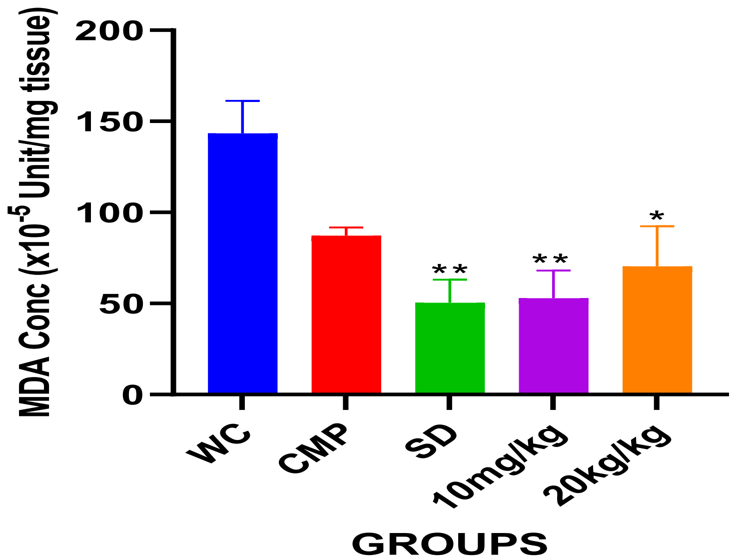


Figure 9: The effect of polyherbal formulated tea tea (*Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca and Thespesia garckeana*) on Malondialdehyde (MDA).

The aqueous extract of polyherbal formulated tea at 10 and 20 mg/kg and sildenafil citrate significantly reduced malondialdehyde (MDA) when compared with normal control and carbamazepine (**p<0.01; *p<0.05). Values were presented as the mean ± S.E.M. n=4.

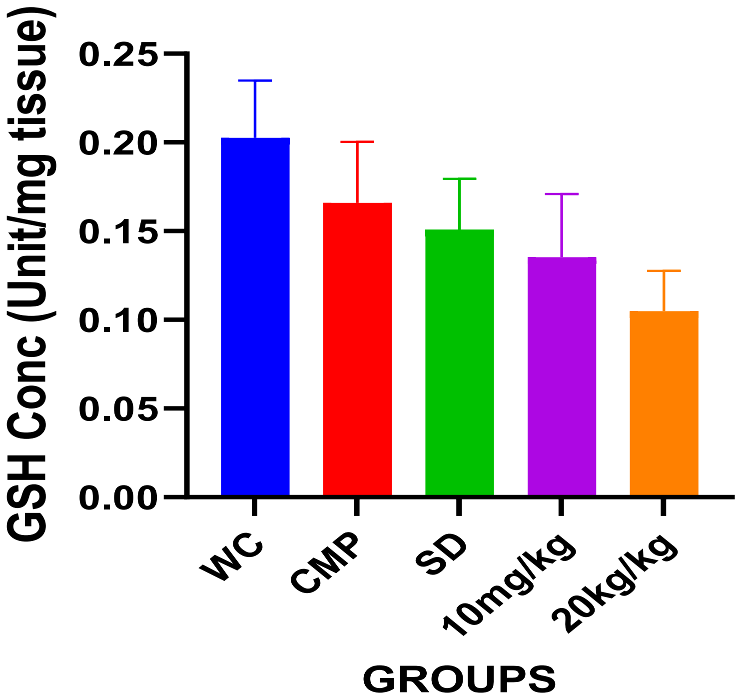
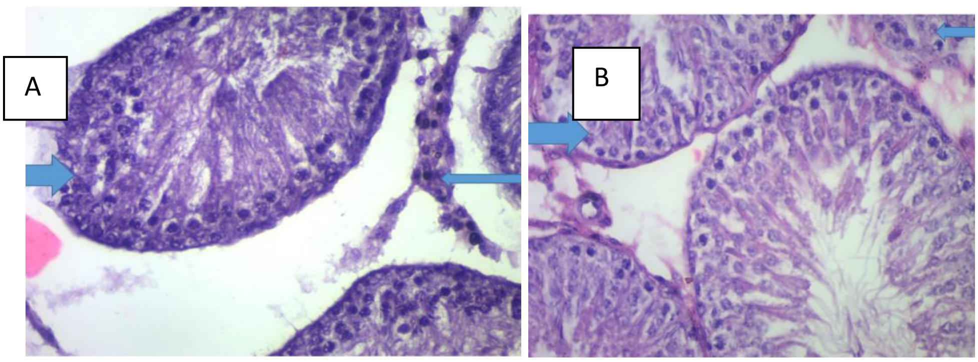


Figure 10: tea (*Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca and Thespesia garckeana* on Glutathione (GSH).

The aqueous extracts of polyherbal-formulated tea at 10 and 20 mg/kg, carbamazepine, and sildenafil citrate have no significant effect on glutathione (GSH) when compared with water control (p > 0.05). Values were presented as the mean ± S.E.M. n = 4.



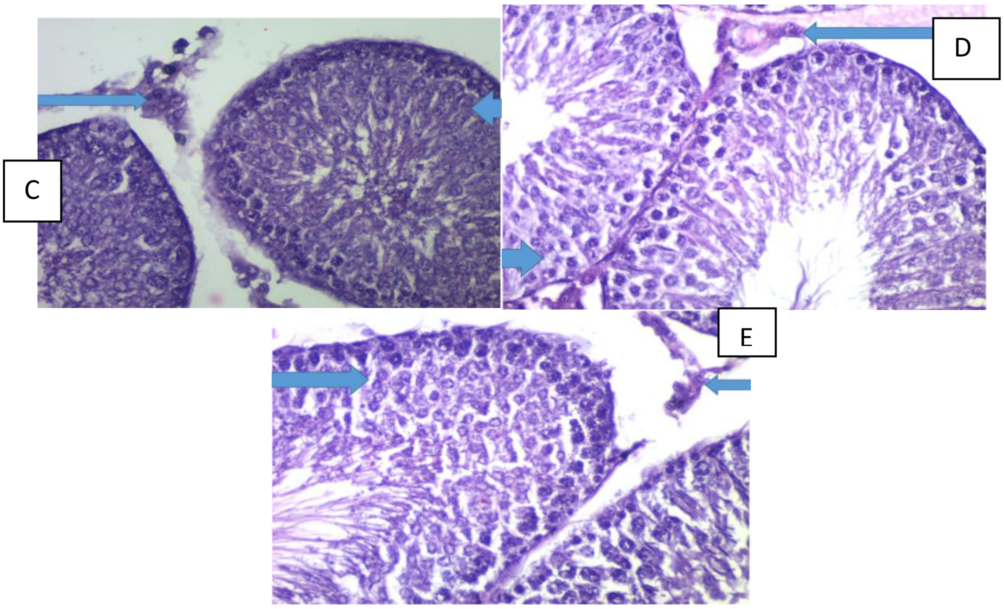


Plate A-Control: A section of the testis shows oval-shaped seminiferous tubules (thick arrow) containing Sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with the presence of Leydig cells in the interstitium (thin arrow). Features are in keeping with normal testicular tissue.

Plate B-Sildenafil Citrate 5 5mg/kg: Section of the testis shows oval-shaped seminiferous tubules (thick arrow) containing Sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with the presence of Leydig cells in the interstitium (thin arrow). Features are in keeping with normal testicular tissue.

Plate C – Carbamazepine 20 mg/kg: A section of the testis shows oval-shaped seminiferous tubules (thick arrow) containing Sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with the presence of Leydig cells in the interstitium (thin arrow). Features are in keeping with normal testicular tissue.

Plate D - 10 mg/kg: A section of the testis shows oval-shaped seminiferous tubules (thick arrow) containing Sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with the presence of Leydig cells in the interstitium (thin arrow). Features are in keeping with normal testicular tissue.

Plate E - 20 mg/kg: A section of the testis shows oval-shaped seminiferous tubules (thick arrow) containing Sertoli cells and sperm cells at different stages of maturation. A thin membrane surrounds the tubules, and the interstitium contains Leydig cells (thin arrow). Features are in keeping with normal testicular tissue**.**

DISCUSSION

In traditional medicine, herbs such as *Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca,* and *Thespesia garckeana* are used to treat various ailments such as malaria, diabetes, neurodegenerative disorders, and gastrointestinal issues (Chinaza *et al.,* 2019; Enwerem *et al.,* 2019; Gondwe *et al.,* 2018; Balunas and Kinghorn, 2005). Researchers have also found them useful in treating infertility by enhancing the motility and viability of sperm. Infertility refers to the inability to conceive after at least one year of unprotected intercourse (Larsen, 2005). In this study, the teas made from *Anthocleista djalonesis, Curcuma longa, Mucuna pruriens, Musa paradisiaca*, and *Thespesia garckeana* at doses of 10 mg/kg and 20 mg/kg did not change the number of sperm counts, while the standard treatment (Sildenafil Citrate 5 mg/kg) increased the sperm count, as shown in Figure 1. Polyherbal tea at 10 and 20 mg/kg and sildenafil citrate significantly increased viable sperm (Figure 5) and reduced non-progressive sperm as well as non-viable sperm (Figures 2 and 6). The ability of a plant to increase sperm viability and progressive sperm motility suggests that the plant possesses properties linked to promoting male fertility (Khaki *et al.,* 2009). The polyherbal-formulated tea's ability to increase sperm viability and progressive sperm motility suggests that the tea possesses properties linked to promoting male fertility. Carbamazepine used as a negative control increased non-progressive sperm and immotile sperm, as seen in figures 3 and 4 (Asadi-Pooya *et al.,* 2015). Carbamazepine (CBZ) is one of the main drugs used to treat epilepsy and neuropathic pain (Boon *et al.,* 2021). Researchers recognise that carbamazepine (CBZ) and its metabolites induce oxidative stress and reactive oxygen species (ROS) (Mittag *et al.,* 2016). Carbamazepine (CBZ) administration reduces sperm concentration, testicular heaviness, testosterone, FSH, and LH hormones (Akorede *et al.,* 2020; Boon *et al.,* 2021; Asadi-Pooya *et al.,* 2015; Kawata *et al.,* 2001). Sildenafil citrate acts by increasing blood flow to the penis during sexual stimulation (Loran *et al.,* 2009). A study shows that *M. pruriens* improved sperm concentration and motility (Ahmad *et al.*, 2008). The ability of *Mucuna pruriens t*o boost sperm concentration and movement is linked to its active ingredients, like alkaloids, coumarins, flavonoids, and alkylamines, which may help enhance the antioxidant levels in men's fertility (Mishra *et al.,* 2024; Ahmad *et al.,* 2008*)*. The phytochemicals in *Thespesia garckeana* have been studied for their potential impact on male fertility, and the plant contains flavonoids, alkaloids, and other compounds that may contribute to improved male reproductive health, including increased testosterone levels. These phytochemicals are believed to play a role in modulating reproductive hormones and supporting healthy testicular function **(**Bukar *et al*., 2021). *Anthocleista djalonensis* also contains flavonoids, alkaloids, phenol and other compounds that may contribute to improved male reproductive health (Popoola, 2020). The phytochemicals in *velvet bean (Mucuna pruriens), Goron Tula (Thespesia garckeana), and Anthocleista djalonensis* might be the reason for the improvement in sperm quality found in this study. Researchers have found that the Velvet bean (*Mucuna pruriens*), the Goron Tula (*Thespesia garckeana*), and the *Anthocleista djalonensis* can help with fertility (Okeke *et al.,* 2019; Maroyi, 2017; Karitha and Thangamam, 2014). McLeod was the first to suggest that oxidative stress may play a role in the aetiology of defective sperm functionality (Aitken *et al.,* 2014). A decrease in seminal plasma antioxidant capacity (TAC) could play a crucial role in the pathogenesis of reduced sperm function. Therefore, we can use the total antioxidant capacity (TAC) as a specific biomarker to gauge oxidative stress in sperm (Pahune *et al*., 2013). At 10 and 20 mg/kg, the aqueous extract of polyherbal-formulated tea significantly increased superoxide dismutase (SOD). It is widely accepted that SOD is the body's first defence against the harmful effects of $\bullet O_2^-$ because it helps convert two $\bullet O_2^-$ molecules into hydrogen peroxide and O_2, which reduces the amount of $\bullet O_2^-$ available, and it plays a significant role in maintaining sperm motility (Öner-İyidoğan *et al*., 2003). Low and diminished SOD activity has been associated with a significant risk of oxidative stress, resulting in diseases such as hypertension, hypercholesterolaemia, atherosclerosis, diabetes, heart failure, stroke, and other cardiovascular diseases (Rosa *et al.,* 2021; Fukai and Ushio-Fukai, 2011). An increase of SOD level by the herbal tea in this study shows that the polyherbal tea may neutralise free radicals that may slow down sperm motility and improve sperm health. In this study, the aqueous extract of polyherbal-formulated tea at 10 and 20 mg/kg significantly increased catalase (CAT). Catalase (CAT) treats many oxidative stress illnesses (Nandi *et al.,* 2019). It is an important enzyme in seminal plasma that aids sperm's antioxidant (Nandi *et al.,* 2019). It contributes to maintaining normal reactive oxygen species (ROS) levels while also protecting sperm from potentially harmful ROS levels. Catalase (CAT) reduces H_2O_2, which is an essential sensor in redox metabolism, to water. H_2O_2 levels are critical to oxidative stress. At 10 and 20 mg/kg, the aqueous extract of polyherbal-formulated tea and sildenafil citrate significantly reduced malondialdehyde (MDA). Sperm cell membranes are vulnerable to peroxidative damage; malondialdehyde (MDA) serves as an index of lipid peroxidation and a marker of oxidative stress, which may be used as a diagnostic tool for analysing infertility in asthenozoospermic patients (Tavilani*et al.,* 2005). Researchers have found that higher levels of malondialdehyde in the blood are linked to lower sperm health, including less viable sperm, reduced movement, abnormal shape, and lower sperm count. The ability of the tea to lower malondialdehyde (MDA) levels indicates that the polyherbal tea promotes sperm health. According to this research, the aqueous extract of polyherbal-formulated tea at 10 and 20 mg/kg has no significant effect on glutathione (GSH). The testicular histopathology has been, for years, one of the crucial investigations in the diagnosis and management of male infertility (Dohle *et al.,* 2012). One of the major challenges with conventional TESE (cTESE) was finding indicators of the likelihood of recovering spermatozoa. Occasionally, circulating hormonal markers, testicular ultrasound, and testicular histopathology have been used (Guler *et al.,* 2016). Histological slides of the testes showed that they were normal (Plates A–E). Malondialdehyde, catalase, and superoxide dismutase significantly influence the potential for sperm fertilisation and male infertility (Tavilani*e et al.,* 2005). A polyherbal tea containing *Anthocleista djalonensis, Curcuma longa, Mucuna pruriens, Musa paradisiaca, and Thespesia garckeana* may boost male fertility, according to this study.

CONCLUSION

The polyherbal-formulated tea has no effect on sperm count, but it does increase sperm motility and viability. It also possesses antioxidant activity.

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Conflict of Interests

There is no conflict between the study's conduct and the authors' interests.

Authors' Contributions

DOU participated in the manuscript's drafting, as well as the study's design and supervision. CCU and JNO actively participated in both the experimentation and the drafting of the manuscript. Before submitting the work, all authors reviewed it and gave their approval.

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