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Original Research Article

THE EFFECT OF *SECURIDACA LONGEPENDUCULATA* CRUDE ROOT-BARK EXTRACT ON SEMEN QUALITY IN GUINEA PIGS

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ABSTRACT

Securidaca longepedunculata is extensively used in complementary and alternative herbal medicine in many African countries. The plant is among the most common treatment options due to its psychotropic properties in treating a wide range of medical conditions such as malaria, tuberculosis, gonorrhoea, diabetes, constipation, syphilis, fungal and skin infections. Additionally, it is also known to alleviate toothaches and headaches, and as an aphrodisiac, improves sexual impotence in males and 'cures' erectile dysfunction. Although its use is widespread, little is known on the mechanism of action in improving sperm quality and thereby its continual traditional application in combating male infertility. This study, therefore, aimed to investigate the effect of *S. longepedunculata* root-bark crude extract on the semen quality of guinea pigs owing to shared similarities in hormonal and particularly immunological responses with spermatogenesis. The phytochemicals of the methanolic crude extract were identified. The extract was administered for 34 days as 50 mg/kg, 75 mg/kg, and 100 mg/kg to groups C, D and E respectively. Treatment using 10mg/kg distilled water and 0.6 mg/kg clomiphene citrate served as negative and positive controls, respectively. Levels of luteinizing and follicle-stimulating hormonal profiles and seminal parameters were analyzed before and after treatment. Eight phytochemicals were detected in the methanolic crude extract. The means of luteinizing hormone before treatment had no significant difference between treatment groups ($P>0.05$. The means of seminal parameters of progressive motility, non-progressive motility, gross motility, and sperm viability between treatment groups were significantly different ($P<0.05$), apart from sperm concentration and immotility. The treatment of guinea pigs with the extract at 75 mg/kg for 34 days increased the levels of luteinizing hormone, sperm motility, and sperm viability. The effects seen at this concentration were similar to those of conventional clomiphene citrate, thereby potentially explaining its continued use as an alternative option for male infertility

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INTRODUCTION

Infertility is the failure of a couple to achieve pregnancy after a year or more of frequent unprotected sexual intercourse [1]. Infertility is considered a major problem in African societies because it remains attached to many cultural norms and beliefs making those that are affected suffer from psychological effects as parenthood in many African homes is

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believed to provide continuity, security, and above all maintenance of the family name [2].

Infertility in males is thought to be a result of problems associated with ejaculation and low sperm count. Ejaculatory dysfunction is a broad term that refers to a man's inability to efficiently ejaculate semen at the moment of sexual climax. This may be caused by blocked ejaculatory ducts and seminal vesicles, infections, or injuries to the genital tract. Low sperm count is a condition where a man has fewer than 15 million/ml of semen. This may be due to hormonal disorders in the pituitary gland, hypothalamus, or testicles, and abnormally shaped sperms among others [1, 3].

Treatment of infertility in men can range from medication therapy to the manipulation of sperm outside the body. Different methods have been used to help with infertility. These include surgery, sperm retrieval techniques and medication such as clomiphene citrate [4]. Because most of these treatments are expensive and for people with failed infertility treatments, many people have opted to seek herbal medicine as an alternative [5].

The use of herbal medicine including *S. longepedunculata* as an element of complementary and alternative medicine in treating infertility is ancient, but is increasingly utilised in both western and southern Africa [6, 7]. However, little is known about how these herbs contribute to sperm quality and subsequently improve fertility. Therefore, this study was intended to give insight into the effects of *S. longepedunculata* crude extract on the semen quality of guinea pigs.

MATERIALS AND METHODS

Plant Identification

The roots of *S. longepedunculata* was freshly collected around 02:33 PM in Mpongwe District, Copperbelt province, Zambia with the help of the local people and accession number UZL 22489 of the specimen was deposited and subsequently identified by botanists at the Department of Biological Sciences at the University of Zambia.

S. longepedunculata Processing and Extraction

The processing and extraction of the plant was carried out as previously described by Chika et al., 2018 [8] with slight modification of concentrating the filtrate in a vacuum drying oven. Briefly, the roots of *Securidaca longepedunculata* was carefully cleaned using clean room temperature distilled water before removing the bark from the root by peeling. The obtained root-bark weighing 562 grams was cut into pieces, air-dried in the shade for 2 weeks (to minimize loss of volatile constituents) before grinding in a blender (logic model). The ground material was kept in the laboratory in a glass jar at room temperature before extraction. The powdered root-bark was extracted by macerating in 20% methanol and kept at room temperature for 48 hours. The extract was then filtered using Whitman filter paper and concentrated in a vacuum drying oven (Binder model) at 49.8 ◦C. The resultant extract was stored in an air-tight container in a refrigerator at 4 ◦C until further analysis to preserve the extract mainly from fungal contamination.

Analyses of Constituents from Crude Extract

The phytoconstituents in the root-bark crude extract were confirmed by phytochemical screening at the University of Zambia, Department of Chemistry. The intelligence test management (Itm) 003il test method was used to test for zinc.

Preparation of Plant Extract for Administration

Preparation of stock solutions of 50 mg/ml, 75 mg/ml and 100 mg/ml respectively was done as previously described by Erhirhie et al., 2014 [9].

Effect of Methanolic Root-Bark Crude Extract

Twenty mature male guinea pigs weighing (600g – 1,200g) body weights were used. The guinea pigs were randomly divided into 5 equal groups (n = 4) namely A, B, C, D and E. They were housed in cages with solid floors and acclimatized for 7 days before commencement of treatment. No food or water was given to the animals for 30 minutes after oral administration of the crude extract to allow for complete absorption of the extract.

2mls of blood samples were collected from the jugular vein using insulin syringes 31G × 5/16" to analyze Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) before and at the end of the experiment. Briefly, the serum used for hormonal profiling was collected from whole blood which was allowed to clot by leaving it undisturbed at room temperature for 4 hours. 5 µl blood serum for each sample was added to the detection buffer and shaken ten times. 75 µl of the mixture was then introduced into the well of the cartilage and then inserted into the iCHROMA II machine (Boditech Med Inc), with present instruction as per manufacturer's manual. This was allowed to run for 3 minutes for the results to be displayed on the liquid crystal display (LCD). The machine was calibrated for guinea pigs before analysis. The laboratory technicians were blinded as the information on the treatment of guinea pigs was withheld.

Group A was given 10 mg/kg distilled water as the negative control group. Group B was given 0.6 mg/kg clomiphene citrate as the positive control group. The treatment groups C, D, and E were given 50 mg/kg, 75 mg/kg, and 100 mg/kg of *S. longependuculata* crude root-bark extract respectively. These dosages were administered orally once a day for 34 days using a syringe. The guinea pigs were weighed after every 5 days to monitor their growth. The guinea pigs were euthanized with 180 mg/kg of sodium pentobarbitone using the method described by Hawkings et al., 2016 [10]. The reproductive organs of the euthanized guinea pigs were then harvested for semen evaluation. Guidelines for ethical conduct in the care and use of animals provided by the National Committee for Research Ethics in Science and Technology were used. Ethical approval to conduct this study was obtained from the University of University of Zambia,

Biomedical Research Ethics Committee (UNZABREC) and the National Health Research Authority (NHRA), reference number 1848-2021 and NHRA00002/24/09/2021, respectively.

Sperm Processing, Motility, Concentration, and Viability

The semen was collected from the caudal epididymis by retrograde flushing method with a 0.5 ml of 0.9 % normal saline using a 2 ml syringe as described by Mwaanga et al., 2014 [11]. Semen analyses were performed according to the World Health Organization protocol on semen analysis [12].

To determine the sperm motility, 5 µl of the semen sample was placed on the makler counting chamber and examined under a light microscope. Portions of the grid were observed and all the motile spermatozoa were counted. Motility was noted as progressive motility (PR), non-progressive motility (NP), and immotility (IM). Gross motility was determined by placing 5 µl of the semen sample on the objective micrometer slide, and was examined under a light microscope.

To determine the concentration of sperm, 5 µl of the semen sample (diluted in the immobilizing solution) was placed on a makler counting chamber, and viewed under a light microscope. Sperm concentration was determined by counting the spermatozoa within any 10 squares of grid. The concentration of spermatozoa was then calculated using the following formula: The number of spermatozoa counted in 10 squares × dilution factor × 10^6 sperm/ml.

To determine the sperm viability, a smear was prepared by mixing 1 drop of the semen sample with 1 drop of 1% Eosin and 2 drops of 10% Nigrosin stain and then air-dried. Using a light microscope, spermatozoa that were not stained were considered to be alive while those which were stained were considered to be dead. The number of spermatozoa alive was divided by the total number of dead spermatozoa, then multiplied by 100 to calculate the percentage of viability as described by Chika et al., 2018 [8]. All magnifications were at X 40.

Statistical Analysis

The data on hormonal profile, sperm motility, sperm concentration, and sperm viability were collected, checked for completeness and consistency. The data were entered into Microsoft Excel then exported to STATA software version 16.0 MP (StataCorp, College Station, Texas 77845 USA) for analysis. Graphical summaries of sperm quality parameters were done using Microsoft Excel. Percentages and frequencies were adopted for summary measures were applicable. All continuous data were assessed for normality using normality plots (q-q plots, histogram, and boxplots). Since the data was skewed, the continuous data were summarised using the median and interquartile range (IQR) and compared using the Kruskal Wallis test. All statistical analysis were conducted at 5% level of significance, thus, only p-values ≤ 0.05 were considered statistically significant.

RESULTS

Identification of Phytochemical Constituents

The zinc levels were 12.5 ug/ml. Gas chromatography-mass spectrometry revealed that the root-bark extract contained 10 constituents with different retention times. Phytochemical screening of the methanolic extracts revealed the presence of phenols, cardiac glycosides, flavonoids, saponins, tannins, alkaloids, steroids and terpenoids as shown in Table 1 with varying color intensities.

Table 1: Phytochemical Screening of the Methanolic Root-Bark Extracts of *S. longependuculata*

Phytochemicals	Inference
Phenols	+
Cardiac glycosides	++
Flavonoids	+++
Saponins	+
Tannins	+
Alkaloids	+
Steroid	+
Terpenoids	+

Key: + + + = High color intensity, + + = Moderate color intensity, += Low color intensity,

Hormonal Effect of Methanolic Extract

The medians of LH at baseline showed no significant difference between treatment groups (P = 0.385). Following treatment, significant differences between the treatment groups were observed (P = 0.020), with the highest level observed in the 75 mg/kg body weight group, 2.43 mIU/mL (IQR, 2.1–3.4), compared to the negative control group 1 (IQR, 1–1.1).

Similarly, there was no significant difference in the median FSH levels between groups before treatment (P=0.642) but after (P<0.0001) treatment. The positive control group given clomiphene citrate showed increased median FSH levels as expected 1.76 mIU/mL (IQR, 1.3–2.23)). The levels of FSH in the negative control group after treatment were identical to the experimental groups given 50 mg/kg, 75 mg/kg, and 100 mg/kg of the extract, respectively.

The Effect of Methanolic Extract on Sperm Parameters

The effect of 50 mg/kg, 75 mg/kg and 100 mg/kg methanolic extract of *Securidaca longependuculata* on sperm quality is illustrated in Figure 1A-F and Table 2.

From Figure 1A, the differences in sperm progressive motility between treatments were significantly different (P=0.012), with guinea pigs treated with 75 mg/kg having the highest median treatment motility of 69.9 (IQR, 56.1–72.7) than the negative control 46.9 (IQR, 24.2–54.6). Progressive motility observed between the treatment group given 75 mg/kg and the positive control group 71.9 (IQR, 52.0–79.7) were comparable. It was observed that the progressive motility of guinea pigs in the 50 mg/kg group 17.52 (IQR, 6.0–31.2) and 100 mg/kg treatment group 11.5 (IQR, 7.4–15.9) had the lowest median motility compared to the negative control

group. Overall, the results obtained showed that the extract at 75 mg/kg had a positive effect on the progressive motility of the sperms similar to that of the positive control.

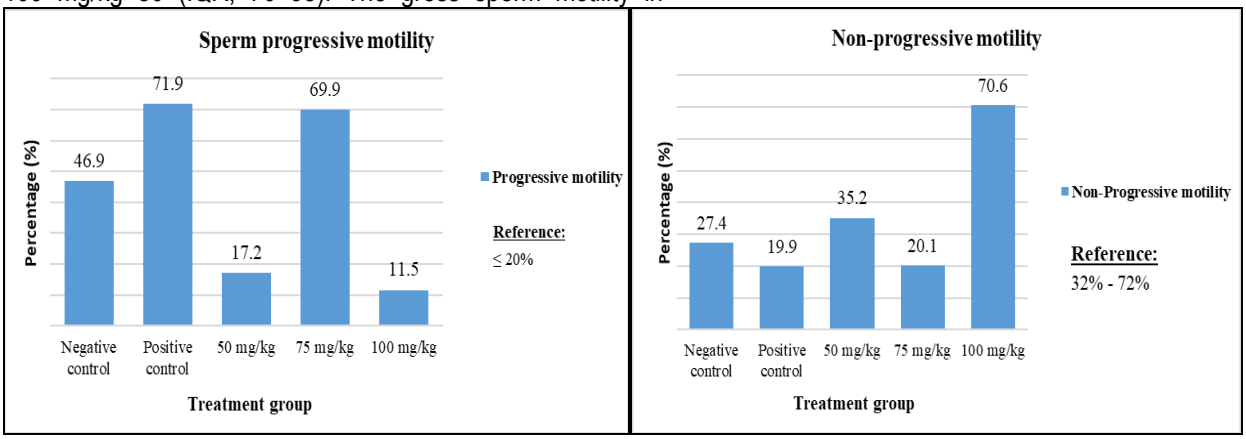
Figure 1B shows significant differences in the medians of non-progressive motility between treatment groups (P=0.042), with all the groups having non-progressive motility above the expected range. Guinea pigs treated with 100 mg/kg of the extract had the highest non-progressive motility 70.6 (IQR, 58.9–73.7) compared to all other groups. The median non-progressive sperm motility in 75 mg/kg treatment group 20.1 (IQR, 19.4–28.0) had essentially the same effect as the positive control group 19.9 (IQR, 12.5–37.6) but much lower compared to the negative control 27.4 (IQR, 12.9–33.7).

Figure 1C shows no significant differences in the median immotility between treatment groups (P=0.126). However, guinea pigs treated with 75 mg/kg had the lowest median number of immotile sperms of 10.8 (IQR, 9.6–19.0) compared to those treated with 50 mg/kg 49.9 (IQR, 28.3–61.3) and 100 mg/kg 18.9 (IQR, 15.1–29.0). Again, the median sperm immotility in the 75 mg/kg treatment group was similar to the positive control 11.5 (IQR, 10.5–13.8).

Figure 1D shows that the gross sperm motility between treatment groups were significantly different (P=0.048), with the highest motility observed in guinea pigs treated with 75 mg/kg 100 (IQR, 95–100) followed by the group treated with 100 mg/kg 80 (IQR, 70–95). The gross sperm motility in guinea pigs given 75 mg/kg of the extract was slightly higher than the group given clomiphene citrate 95 (IQR, 80 – 100). The lowest median gross motility was observed in the group treated with 50 mg/kg 70 (IQR, 60–75) which was similar to the negative control group 70 (IQR, 50–80).

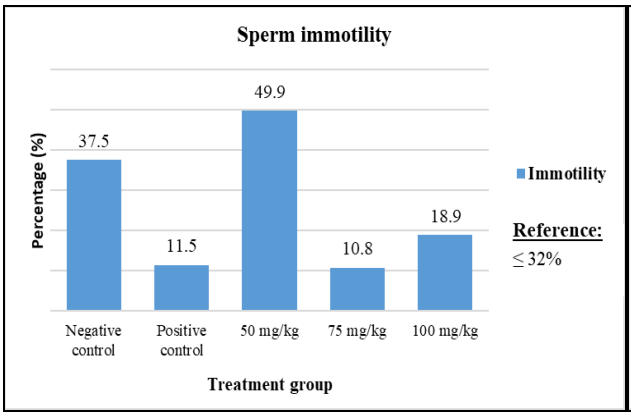
Figure 1E shows no significant difference (P=0.112) in Sperm concentrations between treatment groups. However, the group treated with 75 mg/kg of extract had the highest median sperm concentration 86 (IQR, 85.0–97.3) relative to the group treated with 100 mg/kg 60.7 (IQR, 56.7–67.0) and that treated with 50 mg/kg 49.7 (IQR, 35.3–74.0) of the extract.

Figure 1F shows no significant differences between the treatment groups (P=0.071) in Sperm viability. However, the median sperm viability in guinea pigs in the positive control 85.2 (IQR, 77.7–87.6) was comparable to that in those treated with 75 mg/kg of extract 80.1 (IQR, 78.7–85.7), and was within the expected range (≥58%). The lowest median sperm viability was observed in guinea pigs in the group that was administered 50 mg/kg 51.9 (IQR, 39.6–60.3), and this was below the expected range (≥58%). Overall, the experimental groups, 75 mg/kg and 100 mg/kg showed higher sperm viability compared to treatment at 50 mg/kg body weight and negative control group.



A

B



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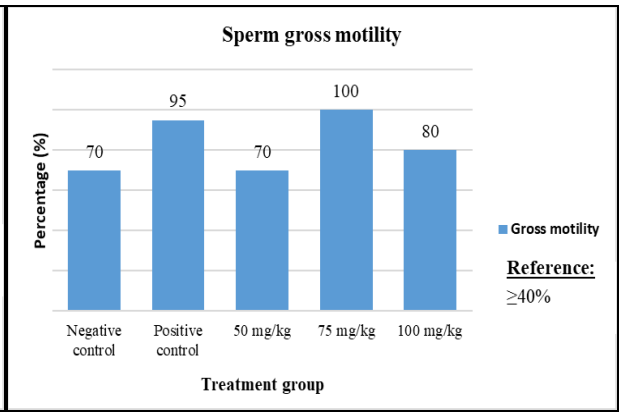

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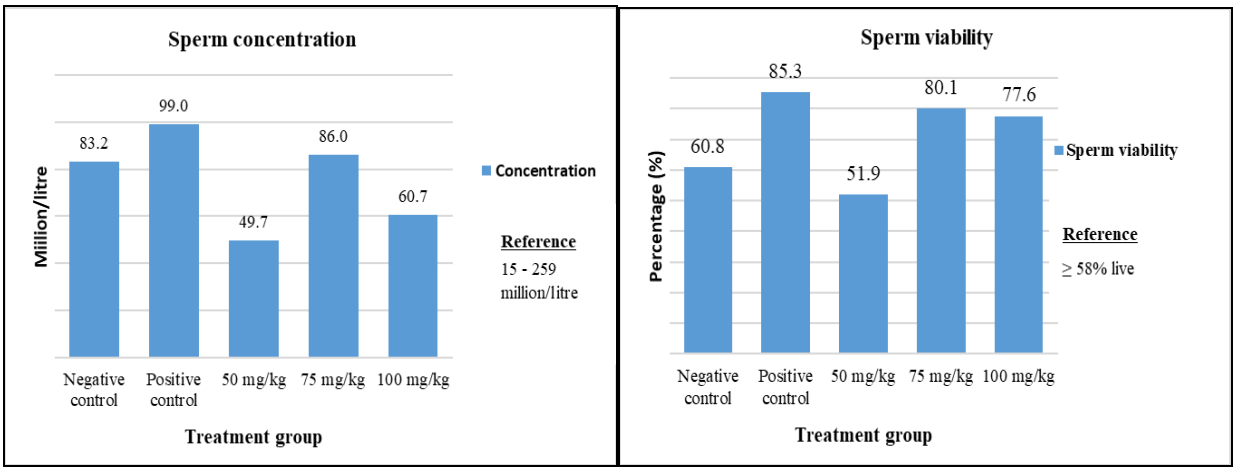

E F

Figure 1: The Effect of *S. Longependuculata* on Sperm Parameters. The panel shows the effect of the crude extract on sperm progressive motility (A), non-progressive motility (B), sperm immotility(C), sperm gross motility (D), sperm concentration (E) and sperm viability (F) after 34 days of treatment.

Table 2: Kruskal Wallis tests for median differences between treatment groups (n=20)

Parameter	Negative control	Positive control	50 mg/kg extract	75 mg/kg extract	100 mg/kg extract	Sig.
LH						
Median (IQR)	1.00 (1.0–1.1)	1.72 (1.3–2.5)	1.00 (1.0 – 1.2)	2.43 (2.1–3.4)	1.00 (1.0–1.7)	**0.020**
FSH						
Median (IQR)	1.00 –	1.76 (1.3–2.2)	1.00 –	1.00 –	1.00 –	**<0.001**
PM						
Median (IQR)	46.94 (24.2–54.6)	71.87 (52.0–79.7)	17.17 (6.0 – 31.2)	69.89 (56.1–72.7)	11.51 (7.4–15.9)	**0.012**
NPM						
Median (IQR)	27.39 (12.9–33.7)	19.95 (12.5–37.6)	35.15 (24.0–49.2)	20.13 (19.4–28.0)	70.56 (58.9–73.7)	**0.042**
Immotility						
Median (IQR)	37.53 (18.0–71.6)	11.47 (10.5–13.8)	49.86 (28.3–61.3)	10.82 (9.6–19.0)	18.88 (15.1–29.0)	0.126
GM						
Median (IQR)	70.0 (50.0–80.0)	95.0 (80.0–100)	70.0 (60.0–75.0)	100 (95.0–100)	80.0 (70.0–95.0)	**0.039**
SC						
Median (IQR)	83.21 (71.2–90.0)	99.0 (66.0–123)	49.67 (35.3–74.0)	86.0 (85.0–97.3)	60.67 (56.7–67.0)	0.112
SV						
Median (IQR)	60.84 (39.5–82.3)	85.26 (77.4–87.6)	51.85 (39.6–60.3)	80.15 (78.7–85.7)	77.57 (73.0–82.4)	0.071

Key: **IQR**=Interquartile Range, **LH**=Luteinizing Hormone, **FSH**=Follicle Stimulating Hormone, **PM**=Progressive Motility, **NPM**=Non-progressive Motility, **GM**=Gross Motility, **SC**=Sperm Concentration, **SV**=Sperm Viability.

DISCUSSION

The application of root-bark extract of *S. longependuculata* is an ancient practice in the absence of access to conventional medicine in many African countries in the treatment of male infertility and so this study elucidated, in part, an understanding into its continued use. The methanolic crude extract content of 12.5 ug/ml of zinc, important in spermatogenesis, was much higher than most leafy vegetables (2 ug/ml) [13]. Furthermore, the crude extract has

evidently been shown to improve the motility of sperms in mammals, sperm viability, and sperm concentration [13]. This is thought to be achieved by maintaining the integrity of the sperm membrane at the end of spermatogenesis hereby, protecting the sperms against free radicles [14]. The amounts found in this plant were also higher than the expected average of 11 µg/ml for successful spermatogenesis. These amounts may most likely contribute to the observed influence in both LH and good sperm quality results observed.

The phytochemical screening reported in this study was consistent with the findings reported by Namadina et al., 2020 [15]. Most of the phytochemicals reported have been known to stimulate the reproductive performance of most mammals by increasing their fertility. For example, saponins have been reported to improve sperm concentration, motility, and viability by increasing the levels of sex hormones, as was observed by the increase in LH levels in this study. LH acts directly on the Leydig cells in the testes to increase the synthesis of testosterone thus, increasing spermatogenesis [16]. Previously a report in Sri Lanka showed that saponins influence LH by increasing its release from the pituitary gland which in turn increases the testosterone levels and also helps in maintaining its levels [17]. Therefore, the increase in LH levels observed in this study may be the reason for the effect the root-bark extract on the seminal quality of the guinea pigs.

Flavonoids are also responsible for improving the semen quality of guinea pigs and increasing LH levels at 75 mg/kg as reported in this study. This phytochemical has been documented to reduce the circulation of Sex hormone-binding globulin (SHBG) which is responsible for binding to male hormones such as dihydrotestosterone and testosterone and carries them throughout the blood consequently improving fertility [18] by the evidenced improved sperm quality parameters as observed in this study. Flavonoids also maintain the androgen synthesis by inhibiting the enzyme aromatase which is responsible in converting testosterone into estrogen thereby, increasing spermatogenesis [19]. Despite having limited financial resources to confirm the levels of testosterone in all the treatment groups, the observed sperm quality gave an indirect indication of high testosterone levels.

Flavonoids and alkaloids have been shown to reduce the plasma concentration of FSH [20] Therefore, their presence can account for the slight reduction in the levels of FSH observed in this study. It is also possible that the extract might have had an effect on the anterior pituitary or the hypothalamus and may have slightly reduced the secretion of FSH since the secretion of FSH is regulated by the gonadotropin-releasing hormone secreted by the hypothalamus [21]. Cardiac glucosides are capable of binding to and inhibiting Na+ /K+ ATPase, a transmembrane protein that controls the active transport of sodium (Na+) and potassium (K+) across the plasma membrane of sperm, thereby possibly aiding sperm motility [22]. Tannins are considered to be natural antioxidants that are involved in eliminating free radicals caused by oxidative stress thereby preventing sperm damage [23]. Steroids act as antiandrogens and they inhibit the enzyme responsible for testosterone biosynthesis as they interact with the steroid hormone receptor [24]. Phenols are also natural antioxidants and they act as free radical scavengers [25, 26] . The presence of these phytochemicals in the methanolic root- bark extract can account for the effect on semen quality observed in this study.

Semen parameters such as sperm motility, sperm concentration, and sperm viability are key indicators of male fertility. Previously, *S. longependuculata* has been shown to improve semen quality of Wistar rats at 50 mg/kg and 75 mg/kg [8]. In this study, an administration of the extract at 75 mg/kg gave better results evidenced by the increased progressive motility, non-progressive motility and gross motility, which were similar to the administration of the conventional clomiphene citrate. The decrease in the number of motile sperms in the group treated with 50 mg/kg of the methanolic extract was however unknown. The decrease in the sperm motility observed in this study can be attributed to the presence of moderate amounts of cardiac glycosides in the methanolic extract, which as explained early would have been capable of binding to and inhibiting Na+/K+ ATPase transmembrane protein that controls the active transport of sodium (Na+) and potassium (K+) across the plasma membrane of sperm thereby, reducing the propulsion of the sperm flagellum [22]. Further experiments may be designed to confirm this assertion.

Non-progressive motility showed a significant difference between the groups with guinea pigs treated with 100 mg/kg having the highest effect, followed by those treated with 50mg/kg. The experimental groups (100 mg/kg and 50 mg/kg) had the highest effect on non-progressive motility compared to other groups. This implied that the sperm motility was poor in these treatment groups as most of the sperm could not make forward progression which is important for successful fertilization. Overall the results showed that the methanolic extract may have increased the movement of the spermatozoa at a medium dose of 75 mg/kg. At low dosages such as 50 mg/kg, there is a possibility of too little extract for any detectable effect. This effect on non-progressive motility may be due to the antioxidant effects of the methanolic extract and also the extract might have been acting on the maturation of the spermatozoa during their movement into the epididymis whose quality was dependent on the dose [27].

Despite having no significant difference in the sperm immotility between the groups, it was observed that the sperm immotility value in the group that received 50 mg/kg was similar to the negative control group which had a higher effect. This implied that the number of sperms that were not making any form of movement was above the expected range (≤ 30 %) thus reducing the sperm motility of sperms in these groups. This increase in sperm immotility at 50 mg/kg is unknown. Lower effect on sperm immotility were

observed in the positive control group and experimental groups that received 75 mg/kg and 100 mg/kg.

The increase in sperm concentration and sperm viability of guinea pigs treated with 75 mg/kg dose may be due in part to the presence of antioxidants such as phenolic compounds, tannins, terpenoids, and flavonoids which possibly were sufficient at this dose to prevent oxidative stress caused by ROS. The aphrodisiac properties and LH level are thought to have increased the levels of testosterone, in turn significantly increasing sperm concentration [28, 29].

CONCLUSION

The treatment of root-bark crude methanolic extract of *S. longependuculata* at 75 mg/kg for 34 days increased the levels of lutenizing hormone, sperm motility, and sperm viability, but surprisingly did not have an effect on the follicle-stimulating hormone. These effects observed at 75mg/kg are due to the phytochemical constituents contained in the crude methanolic extract. The effects at this dose on the seminal parameters of guinea pigs were similar to the conventional treatment drug clomiphene citrate. Therefore, the use of this extract to treat infertility due to hormonal disorders such as low lutenizing hormone and testosterone in the pituitary gland and testicles respectively, is possible and most likely explains its ancient use as part of male infertility treatment plans.

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AUTHORS' CONTRIBUTION

Conceptualization: Margret Singwa. Data curation: Nicholas K Mwale and Margret Singwa. Formal analysis: Andrew Kataba, Nawa Mukena, Nathan Nasson Tembo and Nicholas K Mwale.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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