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Epidydimal Sperm Motility of Cadmium Chloride – Induced Infertile Rats Treated with Aqueous Extract of *Cissus Populnea* Stem

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Abstract

There is a global observed decline in male fertility over the years consequent on various factors affecting spermatogenesis. Some Nigerian males across various geographic and socio-economic strata employ the use of herbals such as Cissus populnea extracts amongst others to manage this and also as herbal aphrodisiacs. This study assessed the spermatogenic effects of aqueous extract of Cissus populnea stem bark.

Cissus populnea stem bark was extracted with water, concentrated and lyophilized. Male wistar rats induced with cadmium chloride were orally administered with 100 mg/kg, 200mg/kg and 400mg/kg body weight of aqueous extract (Cissus populnea) over a 14days period. Sperm of the experimental animals was collected, and the parameters (count, motility, morphology) analyzed.

The oral administration of 400mg/kg C. populnea extract over a 14 days period to male wistar rats induced with cadmium chloride resulted in a four-fold increase in sperm count in the test rats (145±55) compared with the control group (44±17). Testicular histology shows better packed spermatozoa in group of rats treated with Cissus populnea.

The findings suggest that oral administration of Cissus populnea aqueous extract improves spermatogenesis in male wistar rats.

Keywords: Fertility, Cissus populnea, Semen characters, cadmium chloride

Introduction

Infertility can be defined as the inability of a couple to achieve pregnancy after one year of regular and unprotected sexual intercourse (Idrisa *et al.*, 2005). Male infertility refers to a male's inability to cause pregnancy in a fertile female. Male infertility is commonly due to deficiencies in the semen quantity

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and semen quality, and semen quality is used as a surrogate measure of male fecundity. Conditions that can cause male infertility are: infections, hormonal imbalances, diseases, alcohol consumption, cigarette smoking, exposure to chemicals or radiation and so on (Ojekale *et al.*, 2015).

Reproduction (or making a baby) is a simple and natural experience for most couples. However, for some couples it is very difficult to conceive. A man's fertility generally relies on the quantity and quality of his sperm. If the number of sperm a man ejaculates is low or if the sperm are of a poor quality, it will be difficult, and sometimes impossible, for him to cause a pregnancy. Male infertility is diagnosed when, after testing both partners, reproductive problems have been found in the male. Male infertility is usually caused by problems that affect either sperm production or sperm transport. About two-thirds of infertile men have a problem with making sperm in the testis. Either low numbers of sperm are made and/or the sperm that are made do not work properly. Sperm transport problems are found in about one in every five infertile men, including men who have had a vasectomy but now wish to have more children. Blockages (often referred to as obstructions) in the tubes leading sperm away from the testis to the penis can cause a complete lack of sperm in the ejaculated semen. Other less common causes of infertility include: sexual problems that affect whether semen is able to enter the woman's vagina for fertilization to take place (one in 100 infertile couples); low levels of hormones made in the pituitary gland that act on the testis (one in 100 infertile men); and sperm antibodies (found in one in 16 infertile men). In most men sperm antibodies will not affect the chance of a pregnancy but in some men sperm antibodies reduce fertility (McLachlan, 2018).

Male infertility factors contribute to approximately 30% of all infertility cases**,** and male infertility alone accounts for approximately one-fifth of all infertility cases.

Causes of male infertility include; Problems of production of sperm like Oligosospermia, Azospermia, Asthenozospermia and Teratozospermia. Obstruction of the ejaculatory ducts, which can result from infection, can also cause infertility. Alcohol intake and tobacco have been found to be risk factors for infertility in both partners. Causes of female factor infertility include ovulatory disorders, tubal blockage, uteroperitoneal factors like uterine synaechia, uterine fibroid and endometrosis. The role of sexually transmitted infection in causing infertility is well established (Nwajiaku *et al*., 2012).

In Ile-Ife, oligospermia was the commonest sperm abnormality (Ikechebelu *et al*., 2003). This is in contrast with study from Ibadan where the commonest sperm abnormality was
Asthenozoospermia (Adeniji *et al*., 2003). Male factor alone was responsible for infertility in

25% of cases, which compares well with 28.8% in Maiduguri (Idrisa *et al*., 2008), 27%in Ibadan (Adeniji *et al*., 2003), and 22% in Adelaide, Australia (Weiss *et al.,* 1992). Both partners were responsible in 20% of cases and unexplained in 10% of cases (Bayasgalen *et al*., 2004). Female factor alone was responsible for infertility in 45%of the cases. Idrisa *et al* reported a value of
31.3% in Maiduguri (Idrisa *et al*., 2008).

Materials and Methods

Materials

Plants Collection and Authentication

Plants Collection and Authentication

Cissus populnea stem was collected from a local market in Jos, Plateau state. It was identified and authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The plant was authenticated and voucher specimen was deposited under a voucher number (UILH/001/1019) for reference purpose in the department.

Experimental Animals

Twenty-Five (25) Wistar Albino rats (80-120g) of which all were males of Norvegicus strain was obtained from the Small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were housed in well-ventilated cages and allowed to acclimatize to Animal House conditions (i.e. temperature: 28 ^{0}C-31^{0}C; photoperiod: 12 hours' natural light and 12 hours dark; humidity: 50%-55% for 7 days. They were fed with normal rat pellet and tap water.

Chemicals and Drugs

Cadmium chloride (monohydrate) 98% Extra pure was purchased from Sunaf chemical industry in Ilorin, Nigeria. This drug was manufactured in plot No. D-22, Tarapur MIDC, Boisar, Dist. Palghar-401 506. India. Saline solution 0.9% sodium chloride (NaCl) solution

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was prepared manually in the laboratory. All chemicals were prepared in all glass apparatus.

Methods

Plant Preparation

Cissus populnea stem was cut into pieces, oven-dried at 40^{o}C to constant weight and then pulverized using a grinding machine. A known weight of the powder was percolated in 12 litres of distilled water for 72 hours at room temperature with constant stirring. The mixture was thereafter filtered using a Whatman No. 1 filter paper and the filtrate was lyophilized. The resulting extract was weighed and percentage yield calculated. Extract was reconstituted to the required doses.

Analysis of Mineral Elements of *Cissus populnea* Stem

In the mineral determination, wet method was employed in the digestion of the pulverized samples (Ajayi *et al.*, 2006). A representative 5 g of defatted sample was weighed and placed in a digesting flask. This was followed by addition of 20 ml mixture of concentrated nitric acid and perchloric acid (1:1, v/v); with constant stirring on hot plate in a fume cupboard for complete digestion. On cooling, the digests were filtered into 50 ml volumetric flasks, diluted to volume with deionized water and were stored in polyethylene bottle. Atomic Absorption Spectrophotometer (PerkinElmer Model 3110, USA) was used to determine magnesium, manganese, iron, zinc copper and calcium while concentrations of sodium and potassium were determined using Jenway Digital Flame Photometer (PFP7 Model) using the filter corresponding to each mineral element (AOAC, 1999; AOAC 965.17). A certified reference material (CRM) 1570a (spinach leaves) – National Institute of Standard and Technology (NIST), Gaithersburg, MD was used for quality control. Mineral contents of the samples were quantified against standard solution (in a 5% nitric acid matrix) of known concentrations and were run concurrently. All samples and blanks were analyzed similarly according to procedures reported by (Ajayi *et al.* 2006).

Secondary Metabolite Analysis

This screening was done to determine the biologically active compounds present in the plant parts. Procedures were adapted from earlier works for plant analysis as described below and reported by Sofowora (1993) and Trease& Evans (2005).

- **Determination of Alkaloids**

A weighed amount (5 g) of each powdered sample of the plant parts was transferred into a 250 ml beaker. Two hundred ml of 20 % acetic acid was added and then covered to stand for 4 hrs. Filtration was done, and concentration of the extracted content to one quarter of original volume was applied using a water bath. Drop-wise addition of concentrated ammonium hydroxide to the extract followed until the precipitate was complete. The entire solution was allowed to settle and collection of the precipitate was done by filtration (Harborne 1998; Obadoni and Ochuko 2001) and then weighed.

- **Determination of Flavonoids**

To determine the flavonoid content in the stem and root of *C. populnea*, the aluminum chloride colorimetric method was employed. One ml of each plant extract was mixed with 3 ml of methanol, 0.2 ml of 10 % aluminum chloride, 0.2 ml of 1M potassium acetate, and 5.6 ml of distilled water. The entire mixture was allowed to stand at room temperature for 30 min, while the absorbance was measured at 420 nm. The total flavonoid content in each plant part was expressed in terms of standardized quercetin equivalent (mg/g of each extracted compound)

(Aiyegoro and Okoh 2010).

- **Determination of Tannins**

Tannin content was determined using the method outlined. Five hundred mg of the sample was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and then shaken thoroughly for 1 hour in a mechanical shaker. The solution was filtered into a 50 ml volumetric flask and made up to the mark. Five ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M FeCl3 in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Edeoga *et al.*, 2005).

- **Determination of Saponins**

A spectrophotometric method described was used for saponin analysis. One gram of the finely ground sample was weighed into a 250 ml beaker and 100 ml of Isobetyl alcohol was added. The mixture was shaken in a mechanical shaker for 5 hour to ensure uniform mixing. Subsequently, the mixture was filtered through a Whatman No. 1 filter paper into a 100 ml beaker and 20 ml of 40

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% saturated solution of Magnesium carbonate was added. The obtained mixture with saturated MgCO3 was again filtered to obtain a clear colorless solution. One ml of the colorless solution was pipetted into 50 ml volumetric flask and 2 ml of 5 % FeCl3 solution was added and made up to the mark with distilled water and then allowed to stand for 30 min for the development of red color. Standard saponin solutions of 0–10 ppm was prepared from saponin stock solution and each standard solution was treated similarly with 2 ml of 5 % FeCl solution. The absorbances of the sample as well as the standard saponin solutions were read after color development on a Spectronic 2lD Spectrophotometer, at a wavelength of 380 nm, and percentage of saponin was calculated.

- **Determination of Anthraquinone**

Fifty mg of each powdered sample was soaked in 50 ml of distilled water for 16 hours and then heated in a water bath at 70 ° C for 1 hr. The suspension was allowed to cool, after which 50 ml of 50 % methanol was added to it and then filtered. The clear solution was measured with a spectrophotometer at a wavelength of 450 nm and then compared with a standard solution containing 10 mg/100 ml alizarin and 1mg/100 ml purpurin with the absorption-maximum of 450 nm.

- **Determination of Cardiac glycosides**

Buljet's reagent (El-Olemy *et al*., 1994) was used to evaluate the cardiac glycoside content in the examined plant parts. For this purpose, 1 g of each powdered sample was soaked in 100 ml of 70 % alcohol for 2 hours before filtration. Using lead acetate and Na2HPO4 solution, the obtained extracts were purified before the addition of freshly prepared Buljet's reagent. The difference between the intensity of colors of the experimental and blank samples (distilled water and Buljet's reagent) gave the absorbance, which is proportional to the concentration of glycosides.

- **Determination of Cyanogenic glycosides**

Five ml of fine powdered sample of each plant part were weighed into a 250 ml conical flask and incubated for 16 hrs at 38 °C before being extracted with 95 % methanol. The sample was filtered by double layer of hardened filter paper and then distilled with Marham distillation apparatus. The extracted sample was transferred into a low-necked 500 ml flask connected to a steam generator. It was then steam-distilled for 1 hour with sodium bicarbonate solution in a 50 ml conical flask. One ml of starch indicator was added to 20 ml of the distillate and of iodine titrated solution, after which percentage hydrocyanide content was calculated.

- **Determination of Phytosteroids**

For this purpose, the crude extract of each plant material was mixed with 2 ml of chloroform and concentrated sulphuric acid (H2SO4) was added sidewise. The presence of steroids was noticed from the red color produced in the lower layer of chloroform. To confirm further the presence of this phytochemical, another test was performed by mixing each crude extract of the plant materials with 2 ml of chloroform. Two ml of concentrated H2SO4 and of acetic acid were then poured into the mixture and the development of greenish coloration indicated the presence of steroids.

- **Determination of Terpenoids**

The crude extract of each plant part was dissolved in 2 ml of chloroform and evaporated to dryness. Two ml of concentrated H2SO4 was then added to it and heated for 2 minutes. A greyish color indicated the presence of terpenoids. All tests were carried out in triplicate for each plant part, and the results are presented as Mean ± SD. However, the contents were estimated and expressed in mg/g (Micheal and Emmanuel 2012).

Experimental Design

Each of the experimental rats was kept in a standard laboratory setting with a temperature of 25 ± 2 °C and a 12-hour cycle light and dark. The rats were acclimatized in the laboratory for one week. They were given tap water *ad libitum* and fed a normal rodent diet. The rats were subsequently split into five sizable groups at random: fertile rats.

All experimental animals apart from animals in the positive control group were administered intraperitoneally with 0.5g of cadmium chloride dissolved in 500ml of water for 2weeks, while those in the positive control group were given distilled water.

Animal Study and *Cissus populnea* stem extract administration

Twenty-five (25) male albino rats averagely weighing 150.50 ± 10.60 g were split into five groups consisting of five rats each (A - F). The first group of rats were

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normal control rats; they were given water to drink every day; the rats in the second group served as infertile rats. The remaining three groups consisted of infertile rats given 100, 200, and 400 mg/kg body weight of *Cissus populnea* stem extract dosages daily, respectively.

Collection and Treatment of Blood Samples

Animals were sacrificed 24 hours after the last day of treatment. They were anaesthetized with diethyl ether and sacrificed by jugular puncturing. The epididymis was collected into plain sample bottles containing 0.9 normal saline solution.

Microscopic Observation of Sperm Motility

Briefly, the epididymis was dissected out, several incisions were made in it, and it was suspended in 1ml of buffered physiological saline. The preparation was allowed to stand for 10 minutes in an incubator at 37°C to allow sperm swim up. One drop from this preparation was placed on a heated slide for motility estimation. The slides were examined and phase contrast light microscope set at magnifications from 100 to 400. The microscopic field was scanned systematically. An estimate of the percentage motility, motile sperm count and non-motile sperm count was observed.

Statistical Analysis

All data are expressed as mean (of 5 replicates) ± SEM. Data were subjected to statistical analysis using the IBM® Statistical Package for Social Sciences (SPSS) Software Version 20. All significant differences were determined by one way Analysis of Variance (ANOVA). *Post hoc* multiple comparisons were done using Duncan's multiple range test. The significance level was set at $p < 0.05$ (confidence level -95%).

Results

Mineral elements Analysis

Table 1 shows the result of mineral constituents present in *Cissus populnea* stem. It shows the proportion of each mineral. The table shows that the least content is manganese and the most abundant mineral element is zinc.

Secondary Metabolites Analysis

Table 2 shows the results of the analysis for secondary metabolites present in *Cissus populnea* stem. It shows the proportion of each metabolite, phenols and flavonoid are the most abundant secondary metabolites, glycosides and Anthraquinones are the lowest.

Table 1: Mineral Constituents of aqueous extract of *Cissus populnea* stem

Elements	Quantity(mg/kg)
Manganese (Mn)	2.30 ± 0.10
Calcium (Ca)	23.60 ± 0.10
Potassium (K)	56.50 ± 0.04
Magnesium (Mg)	16.30 ± 0.11
Zinc (Zn)	102.00 ± 0.17
Iron (Fe)	82.50 ± 0.16
Sodium (Na)	81.50 ± 0.16

Values are mean of 2 determinations ± SEM

Table 2: Secondary metabolites constituents for *Cissus populnea* stem

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Secondary

Metabolites	Quantity
Saponins (mg/g)	2.85 ± 0.03
Alkaloids (mg/g)	1.62 ± 0.04
Tannins (mg/g)	7.42 ± 0.06
Flavonoids (mg/g)	131.95 ± 0.01
Terpenoids (mg/g)	24.03 ± 0.10
Glycosides (mg/g)	0.99 ± 0.05
Phenols (mg/g)	172.47 ± 0.02
Anthraquinones (mg/g)	0.62 ± 0.10

Values are mean of 2 determinations ± SEM

Motile Sperm Count

The administration of *Cissus Populnea* aqueous extract at the dose of 100mg/kg and 200mg/kg body weight showed no significant difference ($p<0.05$) in the motile sperm count of the experimental animals when compared to the positive control whereas the negative control indicates a significant increase in the motile sperm count. The doses of 400mg/kg body weight also showed a significant increase in the motile sperm count of the animal when compared with the positive control.

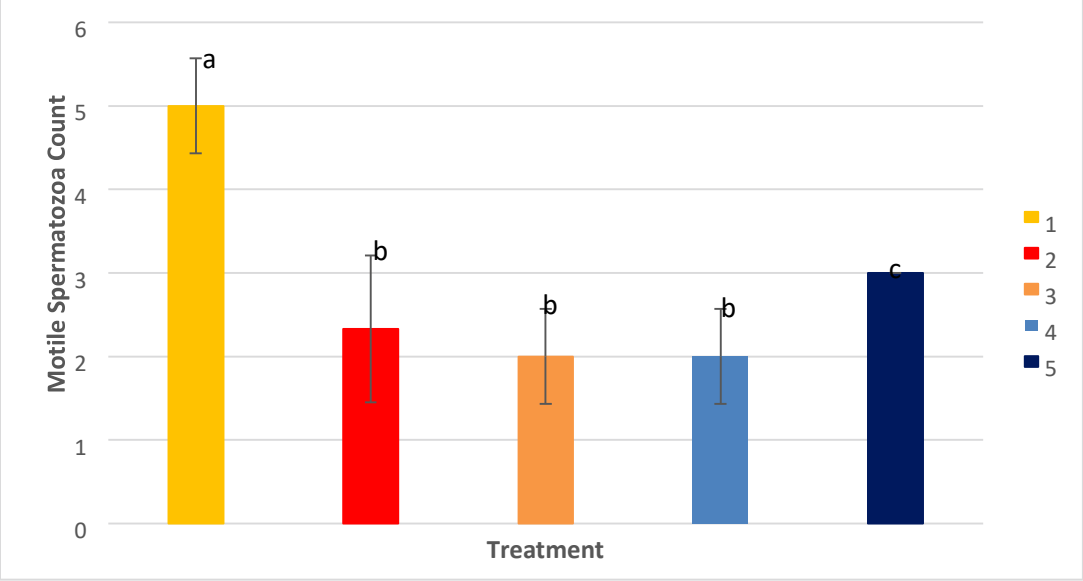


Figure 1: Motile Sperm Count of Cadmium Chloride induced infertility in male rats treated with varying doses of aqueous extract of *Cissus populnea* stem.

None-Motile Sperm Count

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The negative control showed significant high-rate non-motile sperm count of the experimental animals when compared to the positive control. However, dose of 100mg/kg and 200mg/kg shows less significant difference when compared with the positive control.

Administration of 400mg/kg dosage shows vast decrease in the non-motile sperm count when compared with the positive control.

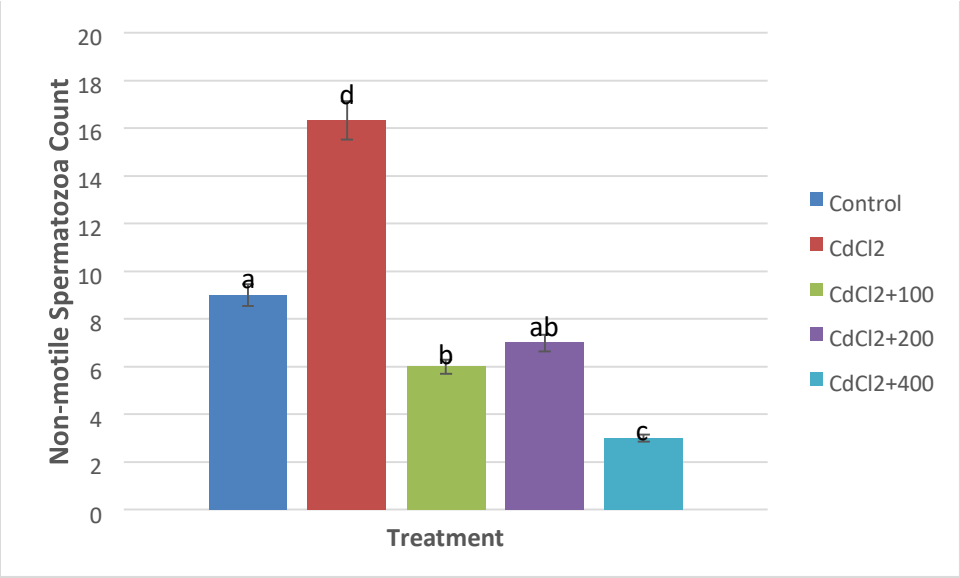


Figure 2: Non-Motile Sperm Count of Cadmium Chloride induced infertility in male rats treated with varying doses of aqueous extract of *Cissus populnea* stem

Percentage Motility

The administration of *Cissus Populnea* aqueous extract at all the varying doses showed a significant increase($p<0.5$) in the percentage motility when compared to that of the positive control carried out on the experimental animal.

Administration of 200mg/kg dose when compared with the positive control has closed values.

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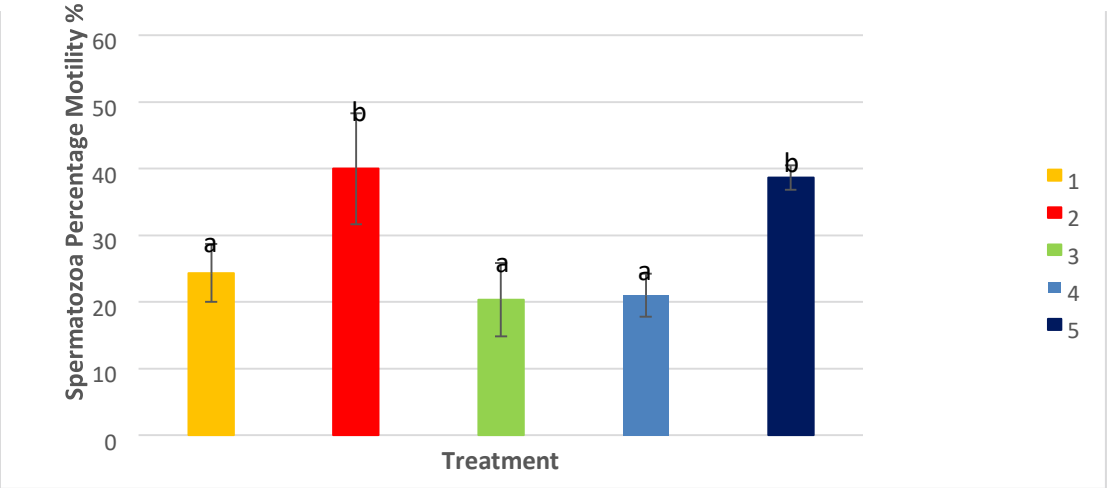


Figure 3: Percentage motility of Cadmium Chloride induced infertility in male rats treated with varying doses of aqueous extract of *Cissus populnea* stem

Discussion

Some studies have shown that the stem bark of *C. populnea* contains secondary metabolites as tannins, flavonoids, saponins, and steroids have been associated with functions related to fertility enhancement potentials (Barry T. (1985); Malini and Vanithakumari, (1991); Ibrahim *et al.*, (1993); Das *et al*., 2004). The fertility ability of saponin rich butanol extracts of *Tribulus terrestris* fruits against 2,3,7,8 tetrachlorodibenzo-p dioxin (TCDD) induced infertility in male albino rats were examined in a study. The saponin caused significant ($P<0.01$) increase in sperm motility, sperm count and sperm morphology (Hemalatha and Rajeswari, 2015).

The most abundant mineral elements in stem bark of *C. populnea* is zinc (table 2). Zinc deficiency impedes spermatogenesis and is the reason for sperm abnormalities and has a negative effect on serum testosterone concentration. Zinc micro element is very essential for male fertility (Fallah *et al.*, 2018).

In this study, there was a significant reduction in the percentage of motile sperm count in group 5 treated with 400 mg/kg body weight of aqueous extract of *Cissus populnea* stem and a favorable increase in the number of sperm cells that travel for fertilization which is similar to the positive control and higher than the other treatment groups. While the group treated with 100mg/kg and 200mg/kg body weight of aqueous extract of *Cissus populnea* stem showed a significant decrease in percentage of motile sperm count. The group 5 treated with 400mg/kg body weight of aqueous extract of *Cissus populnea* stem has the lowest percentage of non-motile sperm count than the other treatments groups.

However, the group treated with 400mg/kg body weight of aqueous extract of *Cissus populnea* stem shows significantly the highest percentage motility when compared with the other treatments. This is a favorable factor that could determine the ability of sperm cell to transport through female reproductive tract and reaches the location of eggs for fertilization.

Conclusion

The effect of aqueous extract of *Cissus populnea* stem on epidydimal sperm motility of infertile male rats showed that the groups treated with different doses of aqueous extract of *Cissus populnea* stem showed similar characteristics but lower than the positive control and/or higher than the negative control groups. Result also showed that there was a favorable decrease in the rate of non-motile sperm count in group 5 treated with 400mg/kg body weight of aqueous extract of *Cissus populnea* stem more than the other treatment groups. This may be due to the fact that the doses for other groups are too low (100mg/kg and 200mg/kg body weight).

Based on the result of this study, 400 mg/kg body weight of aqueous extract of *Cissus populnea* stem administered for ameliorated impairments in the epidydimal sperm motility of cadmium chloride-induced infertile male rats proved effective.

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Therefore, aqueous extract of *Cissus populnea* stem can be employed in the management of male infertility.

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Author Contributions Statement

Omolola Soji-omoniwa: Conceptualized and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials and analysis tools and revised and edited the paper.

Abdujeleel Yusuph: Performed the experiments and redrafted the manuscript.

Saidat Ibrahim: Performed the experiments and redrafted the manuscript.

Akeem Salawu: Analysis and interpretation of data; redrafted the manuscript.

Raphael Olatoye: Analysis and interpretation of data; redrafted the manuscript.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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