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Evaluation of the effect of *Cissus populnea* aqueous root infusion on some testicular function indices and serum hormones of male rats

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Abstract

This study aimed at investigating the effect of aqueous infusion of *Cissus populnea* root on testicular function indices and serum hormones of male Wistar rats. Twenty male albino rats, weighing 130 ±25 g were completely randomized into 4 groups of five each. Animals in groups I to IV received 0.2ml of distilled water, 0.05 ml, 0.1 ml & 0.2 ml/kg body weight of the infusion, respectively. The oral administration was done once daily for 14 days using oropharyngeal cannula. Compared with the control, administration of aqueous infusion of *C. populnea* root at the doses administered resulted in significant (P < 0.05) increase in percentage testesbody weight ratio, serum testosterone, luteinizing and follicle stimulating hormones, acid phosphatase, alkaline phosphatase, testicular cholesterol, protein, sialic acid and glycogen. The results were observed to be 1.73 %, 3.4 ng/ml, 4.2 mlu/ml, 15.5 mlu/ml, 16.8 nM/min/mg protein, 3.39 mmol/ L, 13.58 nM/min/mg protein, 92.75 mg/ml, 1.97 mg/g and 8.38 mg/100mg glucose in the animals respectively compared with the control values of 1.10 %., 1.3 ng/ml, 3.5 mlu/ml, 5.64 nM/min/mg protein, 0.77 mmol/L, 2.58 nM/min/mg protein, 51.58 mg/ml, 1.09 mg/g and 3.13 mg/100mg glucose respectively. The oral administration of aqueous infusion of *C. populnea* root may help to improve fertility in males due to its ability to increase serum testosterone and gonadotropins secretion.

Keywords: *Cissus populnea*, Testicular function indices, Serum hormones, Rats, aqueous infusions.

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Introduction

The testes are two egg-shaped paired organs located between the upper thighs in a skin sack called scrotum. The role of the testes is to secrete the male sex hormone testosterone, which is required for proper physical development in boys, testosterone also maintains libido, muscle strength, and bone density in adults (Schiff et al., 2007). The testis is made up of the tubular compartment (made up of seminiferous tubules, peritubular cells and sertoli cells) and the interstitial compartment (made up of the leydig cells, immune cells, nerves, fibroblasts, loose connective tissue, blood and lymph vessels). The seminiferous tubules are the site for spermatogenesis while the interstitial compartment is the site for steroidogenesis. (Weinbauer et. al.,2010). Several factors can cause damage to the testis and hinder its main functions of spermatogenesis and steroidogenesis while some elements can also enhance its normal functions, one of these includes herbal medicines.

Several herbs are sold commercially in public areas by herb sellers with claims that they increase fertility and enhance reproductive activities. Although some of these herbs have been reported to enhance testicular function indices in rats such as *Eurycoma longifolia, Fadogia agrestis* and *Massularia accuminata* (Yakubu et. al., 2005; Yakubu et. al., 2011; Tambi et. al., 2012), the need for continuous screening of other botanicals for increased reproductive activities cannot be overemphasized.

Cissus populnea Guill. and Per. (Family: Vitaceae/Amplidacea) is widely distributed in West Africa. Some of its local names include 'Okoho' (Idoma, Igala and Igbo tribes of Nigeria), 'Ogbolo or Ajara' by the Yorubas and 'Dafaaraa or Latutuwa' by the Hausas (Burkill, 2000). *C. populnea* is a savannah shrub with a height of about 10 cm and with a diameter of 7.5 cm. It has been linked with numerous therapeutic uses in diverse places. Extracts of *Cissus populnea* have been recognized for antimicrobial properties (Kone et. al., 2004), anti-trypanosomal activities (Atawodi et. al., 2002) and anti-sickling properties (Moody et. al., 2003). It is used for its diuretic properties in Benin Republic while it is used as post-harvest ethnobotanical protectant in Ghana (Belmain et. al., 2000). It is also used to thicken soups, especially among the Idomas of North Central Nigeria. According to previous reviews, the root extract of *C. populnea* has been used for the treatment of wounds, skin diseases and boils (Kone et. al., 2004).

It has been reported also to be used in feeding cattle by the fulanis supposedly increasing milk production (Ojekale et. al., 2006). It is also used in some states in Nigeria for making vegetable soup to stop postnatal bleeding, intestinal parasites and indigestion. It is used as well in the treatment of eye problems that result from attack of black cobra (Soladoye and Chukwuma, 2012). The root cures arrow-poison and also serves as antidote to sore breasts experienced by women at childbearing (Burkill, 2000). The stem bark has been documented to contain flavonoids, carbohydrates, cyanogenic glycosides, tannins, anthraquinones, cardiac glycosides and saponins (Moody et. al., 2003). Infusions of *Cissus populnea* root is popularly sold at car parks and beside the roads to public transport drivers, including bus and bike riders in South-west geria with the indication that it increases libido. This study is therefore aimed at investigating the effect of aqueous infusion of *Cissus populnea* root on testicular function indices and serum hormones of male Wistar rats.

Materials and methods

Plant materials

Cissus populnea root was purchased from local herb sellers in Ilorin, Nigeria. The authentication of the plant was carried out at the Plant Biology Department in the University of Ilorin, Nigeria. A voucher specimen with voucher number UILH/001/1019 was deposited at the herbarium. The herb was prepared in the form of infusions (the plant material was soaked and allowed to stand for 72 hours), 30 g of the root was soaked in 50 ml of distilled water. This was carried out based on the interview with the traditional naturopaths on the preparation of the sample.

Experimental animals and infusion administration

Twenty male albino Rats (Rattus norvegicus) of Wistar strain weighing 130 ±25 g were obtained from the Animal Holding Unit, Biological Sciences Department, Landmark University, Nigeria. The rats were housed in clean cages that provided free access to rat pellets and tap water after their daily doses throughout the period of extract administration. Before the experiment commenced, the animals were acclimatized for two weeks.

The experimental rats were completely randomized into four groups of five animals each. The animals in groups I to IV orally received 0.2 ml of distilled water, 0.05 ml, 0.1 ml & 0.2 ml/kg body weight of *Cissus populnea* aqueous root infusion respectively. Administration of infusion was done once a day, orally for duration of 14 days between the hours of 09:00 09:45 an oropharyngeal cannula. The weight of the animals were taken and recorded twice a week and the animals were sacrificed on day 15.

The animals were sacrificed twenty-four hours after their last dose, blood samples were collected, their testes were excised and homogenates obtained using standard procedure (Sahoo, 2013).

Determination of some reproductive hormones

The assay kits used for the determination of cholesterol was a product of Agappe Diagnostics Ltd, Switzerland while the assay kits for the hormonal assays (testosterone, follicle stimulating hormone and luteinizing hormones) were products of Monobind Inc. Lake Forest USA.

The concentration of testosterone in the serum as well as serum luteinizing and follicle stimulating hormones were accessed using the method described by the manufacturer in the instruction manual.

Estimation of biochemical parameters

These parameters were quantitatively determined in the testicular supernatant using standard procedures. The concentration of total protein described by Gornall et al. (1949), total cholesterol based on the reaction outlined by Fredrickson et al., (1967), glycogen (Kemp et al., 1954), and sialic acid (Warren, 1959) in the testes were estimated. Wright et al., (1972a, 1972b) described the procedures for the assays to evaluate the specific enzymatic activities of alkaline phosphatase (ALP) and acid phosphatase (ACP).

Ethics

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed

Statistical analysis

The data acquired from this study were expressed as mean ± standard error of mean and were analyzed using a one-way analysis of variance (ANOVA) with multiple comparisons. Values at $p < 0.05$ were considered statistically significant.

Results

Table 1 shows the effect of Cissus populnea aqueous root infusion on rats' body weight during the 14 days administration. The trend showed that the Cissus populnea aqueous root infusion brought about general increase in the weight of the animals across all groups, this increase was observed to be significant on day 14 in animals that received 0.05 and 0.1 ml/kg body weight of infusion but it was not significant in animals that received the highest dose 0.2 ml/kg body weight.

Table 1: Effect of Cissus populnea aqueous root extract on body weight (n = 5)

GROUPS	DOSE (ml/kg-bt)	DAY 1	DAY 7	DAY 14
CONTROL	0.2ml of D$_2$O	114 ± 1.4	124 ± 1.8	124 ± 1.5
GROUP	0.1	134 ± 2.6	146 ± 2.6	157 ± 2.6
GROUP	0.05	151 ± 5.1	168 ± 1.6	181 ± 1.1
GROUP	0.2	147 ± 7.2	149 ± 2.2	169 ± 0.2

n = number of rats used; = distilled water at $p < 0.05$.

As shown in Figure 1, at the various doses of the aqueous infusion of *Cissus populnea* root, there was an increase in the percentage testes-body weight from 1.29 % to 1.73 % when compared with the control value of 1.10 %. This was observed to be in a dose dependent manner. The animals displayed a significant increase ($P < 0.05$) in their percentage testes-body weight ratio in the groups that received 0.1 ml and 0.2 ml/kg of the aqueous infusion when compared with the control.

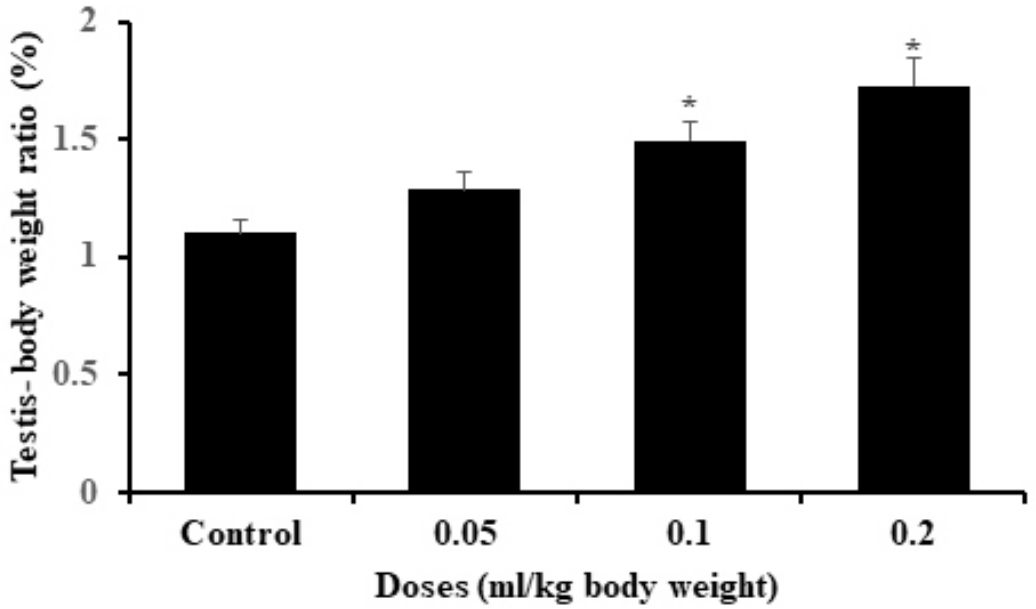

Figure 1: The effect of aqueous infusion of *Cissus populnea* root on percentage testes-body weight ratio. *Means significantly different at $P < 0.05$

From Figure 2, oral administration of aqueous infusion of *C. populnea* root on serum testosterone at dose range of 0.05, 0.1 and 0.5 ml/kg resulted in an increase which ranged from 2.1 to 3.4 ng/ml when compared with the control which was 1 ng/ml. All the increases were observed to be dose related. The 0.05 ml/kg body weight of aqueous infusion of *Cissus populnea* increased the serum testosterone level by about double the control values whereas the increase produced by the 0.1 and 0.2 ml/kg body weight was triple the control value. Significant increase ($P < 0.05$) in the level of serum testosterone compared with the control was observed across all the treated animals, this was observed to be in a dose dependent manner.

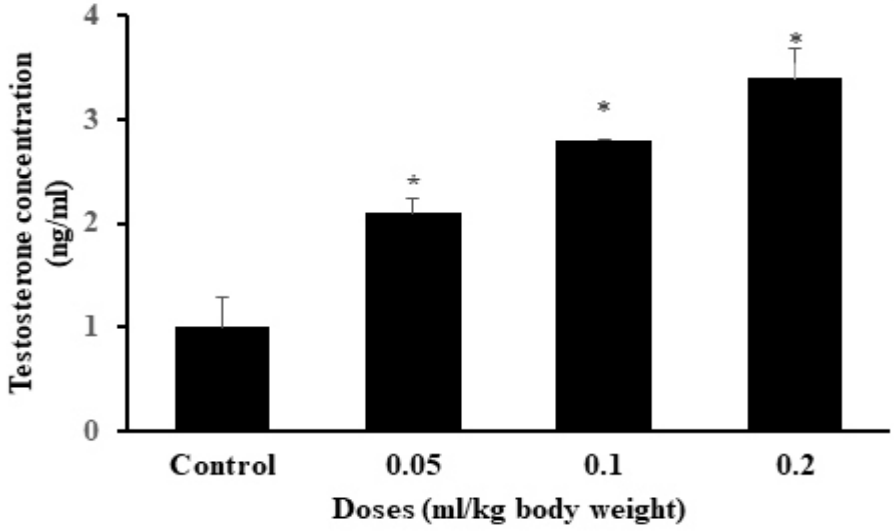

Figure 2: The effect of aqueous infusion of *Cissus populnea* root on serum testosterone concentration. *Means statistically significant at $P<0.05$

The effect *C. populnea* aqueous root infusion on serum luteinizing hormone is shown in Figure 3. It was observed to increase from 2.7 to 4.2 mlu/ml across all groups compared with the control which was 1.3 ng/ml. The 0.05 ml/kg body weight of aqueous infusion of Cissus populnea increased the serum luteinizing hormone concentration by about double the control value whereas the increase produced by the 0.1 and 0.2 ml/kg body weight was triple the control value. Therefore the administration of aqueous infusion of *Cissus populnea* root for 14 days significantly ($P < 0.05$) increased the serum level of luteinizing hormone in the animals, dose dependently, when compared with the control.

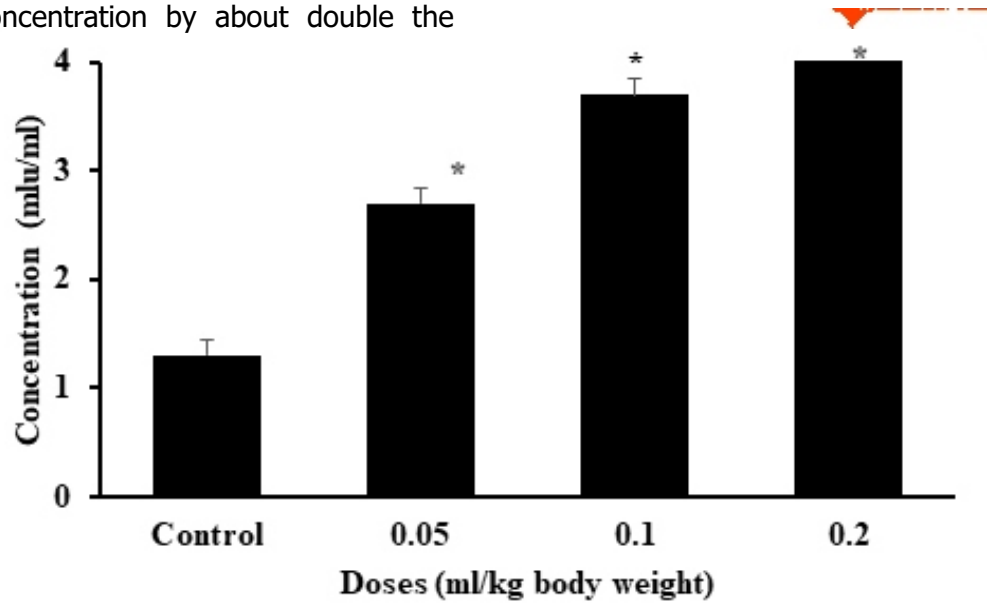

Figure 3: Effect of aqueous infusion of *Cissus populnea* root on serum luteinizing hormone concentration. *Means significantly different at P<0.05

Figure 4 shows the effect of aqueous infusion of *Cissus populnea* root on serum follicle stimulating hormone concentration. The serum follicle stimulating hormone concentration was observed to range from 9.4 to 15.5 mlu/ml at the various doses administered. The highest value for the serum follicle stimulating hormone concentration was observed in animals that received the aqueous infusion concentration of 0.1ml/kg body weight which was 15.5 mlu/ml while animals that received 0.05 ml/kg body weight of infusion had the lowest value which was 9.4 mlu/ml. The animals displayed a significant increase ($P < 0.05$) in FSH levels across all the treated groups when compared with the control.

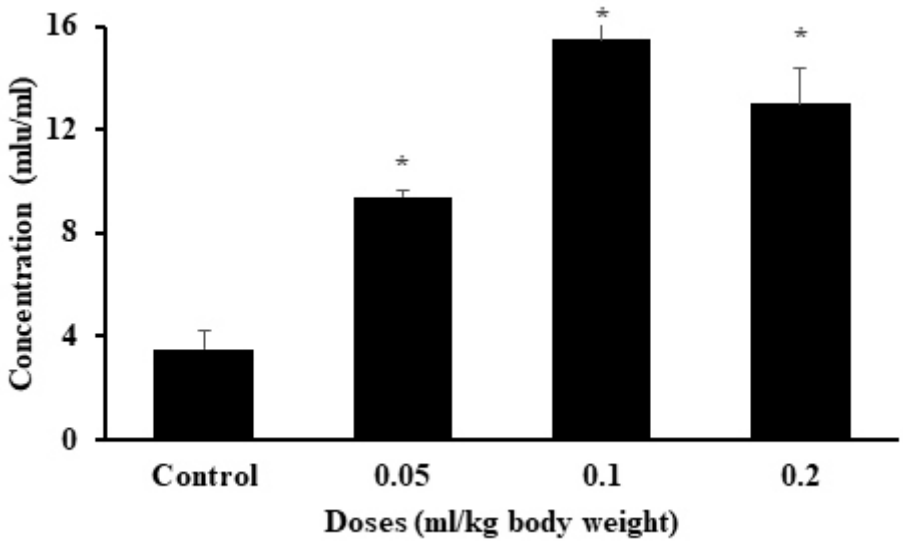

Figure 4: Effect of aqueous infusion of *Cissus populnea* root on serum follicle stimulating hormone concentration. * Means significantly different at p<0.05.

As shown in Figure 5, the effect of oral administration of aqueous infusion of *C. populnea* root on testicular acid phosphatase activity at the doses of 0.05, 0.1 and 0.2 ml/kg resulted in an increase which ranged from 7.7 to 16.8 nM/min/mg protein when compared with the control which was 5.64 nM/min/mg protein. All the increases observed were dose related. The 0.1 ml/kg body weight of aqueous infusion of *Cissus populnea* increased the acid phosphatase activity by about double the control value whereas the increase produced by the 0.2 ml/kg body weight was triple the control value. Significant increase ($P < 0.05$) in the level of testicular acid phosphatase compared with the control was only observed in animals that received 0.1 ml/kg and 0.2 ml/kg body weight of the infusion.

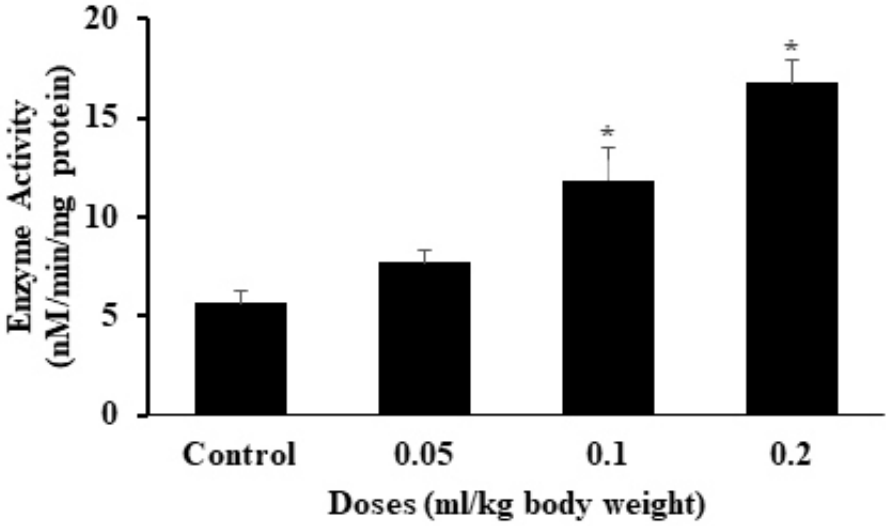

Figure 5: Effect of aqueous infusion of *Cissus populnea* root on acid phosphatase activity. * Means significantly different at $p<0.05$

Figure 6 shows the effect of oral administration of aqueous infusion of *C. populnea* root on testicular alkaline phosphatase activity at the doses of 0.05, 0.1 and 0.2 ml/kg. This resulted in an increase when compared with the control which was 2.58 nM/min/mg protein, this increase was observed to range between 12.10 nM/min/mg protein and 13.58 nM/min/mg protein. All the doses of aqueous infusion of *Cissus populnea* administered increased alkaline phosphatase activity by minimum six-times the control value. Significant increase in testicular alkaline phosphatase level compared with the control was observed across all the treated animals.

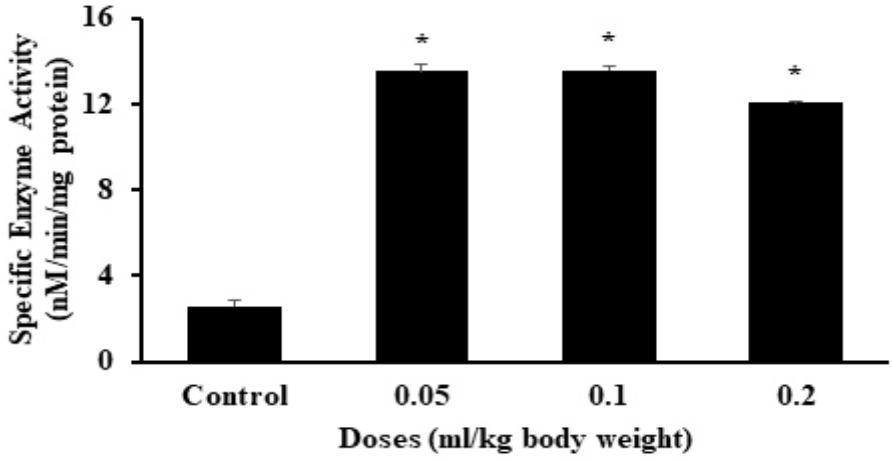

Figure 6: Effect of aqueous infusion of *Cissus populnea* root on alkaline phosphatase activity. * Means significantly different at $P<0.05$

As shown in Figure 7, the result of oral administration of aqueous infusion of *C. populnea* root on testicular protein content at doses given (0.05, 0.1 and 0.2 ml/kg) was observed to be 69.75, 92.80, 78.09 mg/ml when compared with the control which was 51.58 mg/ml. The highest increase observed for the testicular protein content was detected in the animals that received infusion concentration of 0.1ml/kg body weight while animals that received 0.05 ml/kg body weight had the lowest values of testicular protein content. Significant increase ($P < 0.05$) in the testicular protein was observed across all treated groups compared with the control.

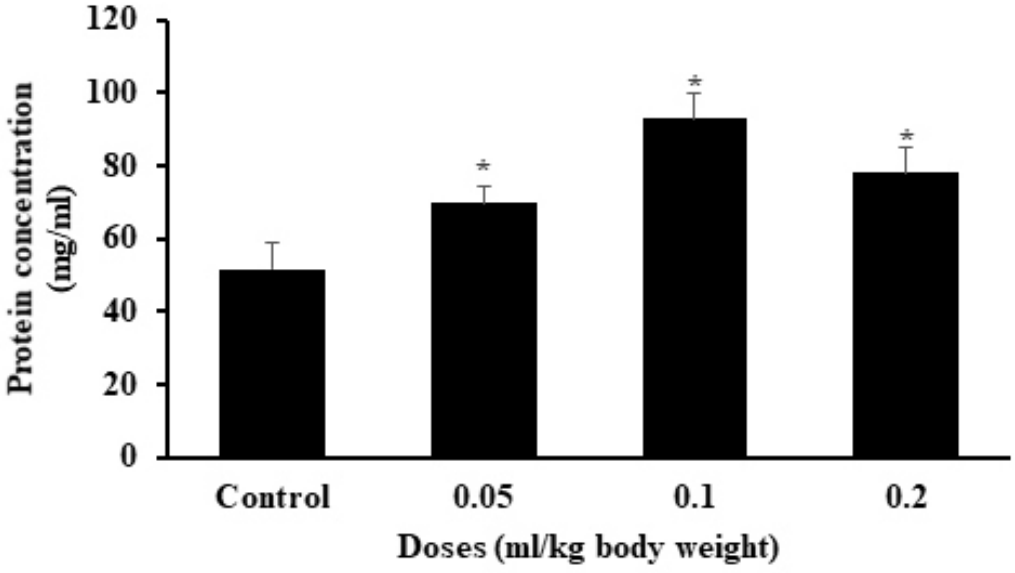

Figure 7: Effect of aqueous infusion of *Cissus populnea* root on testicular protein content.
*Means significantly different at P<0.05

In figure 8, the effect of oral administration of aqueous infusion of *C. populnea* root on testicular glycogen at dose range of 0.05, 0.1 and 0.2 ml/kg is shown. An increase in testicular glycogen level was observed from a control value of 3.13 mg/100mg to 5.33, 7.23 and 8.38 mg/100mg respectively after 14 days of administration. The highest increase observed was detected in the animals that received infusion concentration of 0.2 ml/kg body weight while animals that received 0.05 ml/kg body weight had the lowest values of testicular glycogen content. The increase observed was significant ($P < 0.05$) across all treated groups compared with the control.

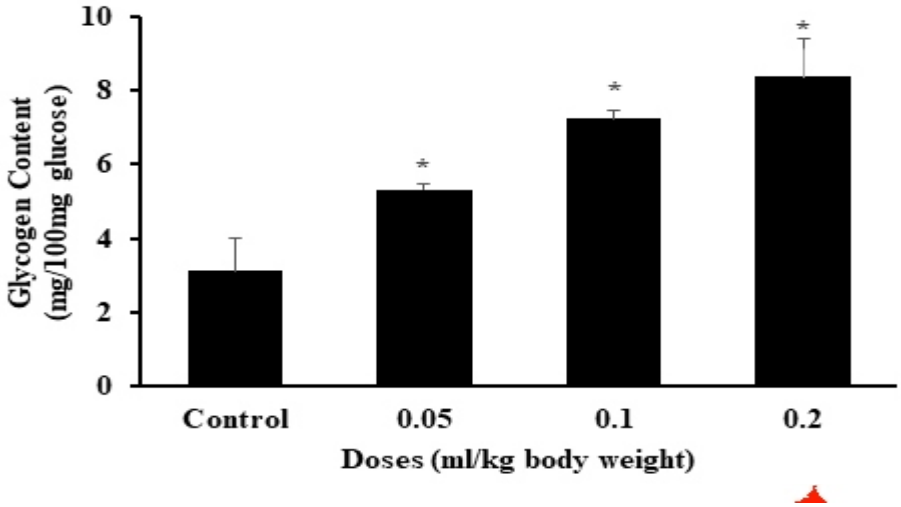

Figure 8: Effect of aqueous infusion of *Cissus populnea* root on testicular glycogen.
*Means statistically significant at P<0.05

The result of *C. populnea* aqueous root infusion on testicular sialic acid is shown in figure 9. In animals that received doses of 0.05, 0.1 and 0.2 ml/kg body weight of infusion, an increase in testicular sialic acid level was observed across the groups ranging from 1.78 to 1.97 mg/g while the control value was 1.09 mg/g. Compared with the control, this increase was observed to be significant ($P < 0.05$) in all treated groups.

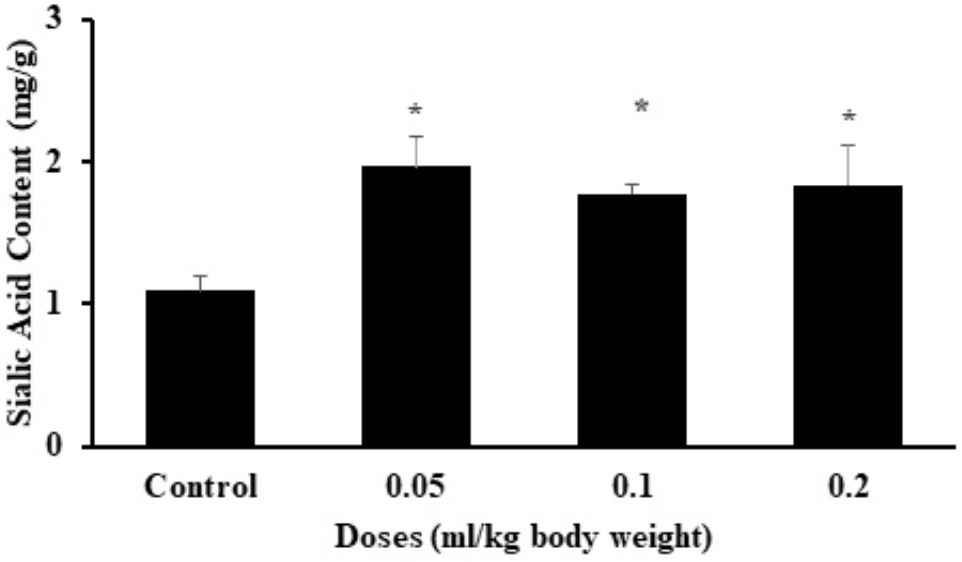

Figure 9: Effect of aqueous infusion of *Cissus populnea* root on testicular sialic acid concentration. *Means significantly different at $P < 0.05$

As shown in figure 10, oral administration of *C. populnea* aqueous root infusion on testicular cholesterol resulted in an increase from the control value of 0.77 mmol/L to 1.63, 2.14 and 3.39 mmol/L. The highest increase observed for the testicular cholesterol was detected in animals that received infusion concentration of 0.2 ml/kg body weight while animals that received 0.05 ml/kg body weight had the lowest value. Significant increase ($P < 0.05$) in the level of testicular cholesterol across all the treated groups was observed when compared with the control, this was found to be in a dose dependent manner.

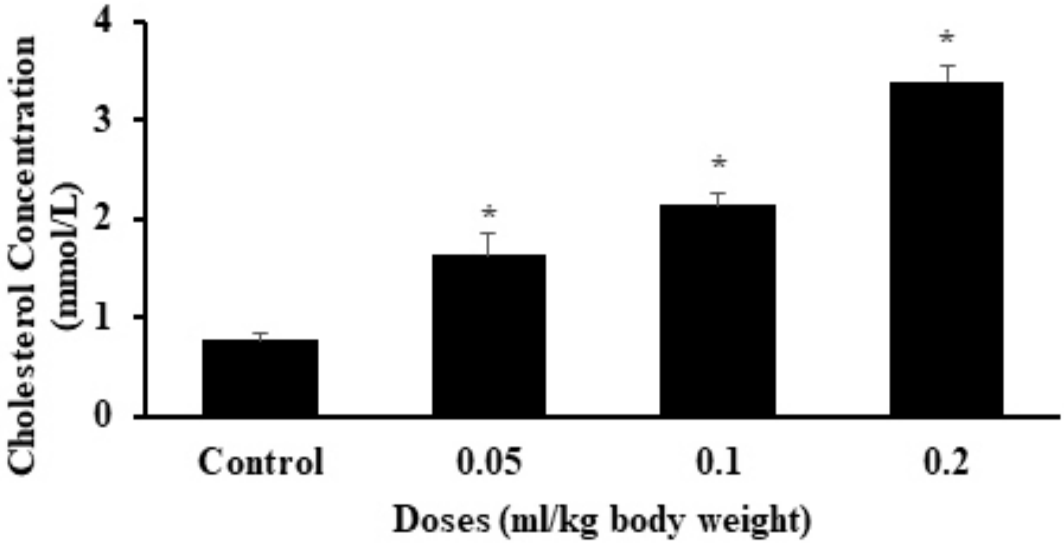

Figure 10: Effect of aqueous infusion of *Cissus populnea* root on testicular cholesterol concentration. *Means significantly different at $P < 0.05$

Discussion

The observed increase in the testis-body weight ratio and final weight after the administration of aqueous infusion of *Cissus populnea* root shows that the animals were generally healthy and experienced growth, the extract did not negatively impede the weight of the animals. The observation regarding increase in testis-body weight ratio is similar to earlier report by Olaolu et. al. (2015) where administration of aqueous leaf extract of *Cissampelos mucronata* was observed to increase the testis-body weight ratio.

The significant rise in the level of testosterone observed in this present study at all the doses suggests that the administration of *Cissus populnea* root infusion enhanced its synthesis by the leydig cells, this should result into an improvement in the reproductive activities, sexual drive and fertility in the male rats. It agrees with the report of Tambi et. al. (2012) where standardized water-soluble extract of *Eurycoma longifolia* was used as testosterone booster for managing men with late-onset hypogonadism. The observed increase in the level of testosterone also correlates with an upsurge in cholesterol (the starting material for and rogen synthesis) in this present study. Testosterone is the primary male sex hormone. It is a steroid hormone required for spermatogenesis (Tuck and Francis, 2009).

LH is crucial for stimulating the production of testosterone by leydig cell, it is produced by the pituitary gland in response to the production of GRH (gonadotropin releasing hormone) by the hypothalamus. Thus, the observed increase in the serum luteinizing hormone level at different doses of aqueous infusion of *Cissus populnea* root may indicate an enhanced hypothalamic-pituitary function of the rats. The finding in this study is related to that of Yakubu et. al. (2011) where an increase in serum luteinizing hormone was observed after *Massularia acuminate* aqueous root extract was administered to male Wistar rats resulting in improved fertility rate.

Increase in the synthesis of follicle stimulating hormone (FSH) leads to an effective gonadal function which also indicates increased sperm production and maturation (Boulpaep and Boron, 2005). The significant increase in the level of serum FSH at all doses after the administration of the aqueous infusion of *Cissus populnea* root may result into enhanced reproductive ability in rats and this also implies enhanced sperm maturation as a result of the normal functioning of the sertoli cell (Boulpaep and Boron, 2005). The findings in the present study is similar with previous report of Egba et al. (2014) where the effect of oral administration of aqueous extract of *Newbouldia leavis* leaves showed an increase in serum FSH level of female Wistar rats.

Acid phosphatase ACP boosts spermatogenic activity as well as the material interchange between the sertoli cells and the germinal cells (Tietz, 1995). The significant increase observed with the administration of aqueous infusion of *Cissus populnea* root helps improve material interchange between the sertoli cells and the germinal cells. The result obtained for acid phosphatase activities in this study is in contrast to the report of Nurudeen and Ajiboye (2012) after the administration of aqueous root extract of *Lecaniodiscus cupanioides* which led to restoration of the alterations in testicular parameters of sexually impaired rats.

Alkaline phosphatase ALP mobilizes metabolites usually carbohydrates and lipids which are used by the sperm cell in the seminal fluid (Yakubu et. al., 2008). The rise in the enzymatic activity of ALP observed after administration of aqueous infusion of *C. populnea* root may indicate enhanced mobility of carbohydrates and lipid metabolites. The result obtained for alkaline phosphatase activities in this current study is in line with that of Yakubu and colleagues (2008) where he reported the effects of oral administration of aqueous extract of *Fadogia agrestis* stem on testicular function indices of male rats.

Testicular protein is dependent on testosterone action and is also important for sperm maturation (Gupta et. al., 2004). An enhancement in the synthesis of protein detected in this present study may increase sperm function and maturation. The pattern of dose-dependent increase in testicular total protein content is comparable to the result of previous study by Yakubu and co-workers (2007) after aqueous extract of *Chromolaena odoratum* was administered to male rats for 14 days.

Testicular glycogen reserve is the sertoli cells and spermatogonia, providing carbohydrate and energy during the development of the male gonads and for seminiferous tubular cells (Yakubu et. al., 2008; Nurudeen and Ajiboye, 2012). Therefore, the increase in testicular glycogen by the aqueous infusion of *C. populnea* root suggests an enhanced energy release which is important for spermatogenesis (Yakubu et. al., 2007). The result obtained in this study is similar to previous report by Olaolu et. al. 2015, where administration of *Cissampelos mucronata* aqueous leaf extract was observed to bring about an increase in testicular glycogen level.

Sialic acid performs the function of a lubricant which facilitates sperm motility and reduces friction among spermatozoa and subsequently enables their upward movement within the testes lumen and during their transit through the epididymis and the vagina (Chinoy and Bhattachary, 1997). The increase in testicular sialic acid by the aqueous infusion of *C. populnea* root may have positive effect on the structural integrity of acrosomal membrane which ultimately may affect the metabolism, motility and fertilizing capacity of spermatozoa (Chinoy and Bhattachary, 1997). A similar result was reported on the effect of aqueous root extract of *Lecaniodiscus cupanioides* restoring the alterations in testicular parameters of rats by Nurudeen and Ajiboye (2012).

Cholesterol serves as precursor to steroid hormones especially those involved in steroidogenesis which is required for increased testicular function (Nurudeen and Ajiboye, 2012). Significant increase in testicular cholesterol level indicates enhancement of steroidogenesis, which leads to increased testosterone, FSH and LH concentration (Nurudeen and Ajiboye, 2012). The result obtained in this study is in line with that of Olaolu et. al. 2015, where administration of *Cissampelos mucronata* aqueous leaf extract was observed to result in an increase in the cholesterol level of the male animals used.

Testes-body weight ratio and testicular function indices (sialic acid, alkaline phosphatase, acid phosphatase, protein, glycogen, and cholesterol concentration) are all closely regulated by androgen concentration (Gupta et. al., 2002; Kamatchouing et. al., 2002; Watcho et. al., 2004). Since androgens, notably testosterone, are produced by the testes, the evaluation of these functional parameters serve as useful indices of androgenicity and indirect assessment of fertility in male rats. The results of this current study implies that aqueous infusion of *C. populnea* root does not have detrimental effect on the weight of persons that use them. It also has the ability to increase testosterone level, which is the primary androgen in humans possibly through increase in the level of its precursor, cholesterol, as well as the gonadotropins and other testicular function indices. This will subsequently lead to an overall enhancement in fertility in humans.

Conclusion

The result of this present study has shown that aqueous infusion of *C. populnea* root (Guill and Perr) at the various doses administered enhanced the overall testicular function indices in the animals when compared with the control. It may therefore be inferred that aqueous infusion of *C. populnea* root may help to improve fertility in male rats due to its ability to increase androgen level, gonadotropins and other testicular function indices which could boost reproductive activity in males.

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