ORIGINAL ARTICLE

Discovery Phytomedicine 2020, Volume 7, Number 2: 58-64

Anti-fertility properties of *Cissus rotundifolia* (Forssk.) Vahl. Extract using female Wistar rats

Anita Mary Mziray,[1*] Charles Irungu Maina, Catherine Kaluwa Kaingu[2]

ABSTRACT

A third of the worldwide disease burden among women in their reproductive age (15-45 years) is associated with sexual and reproductive complications. The developing world experiences an unmet contraceptive demand that affects nearly 200 million women. The high rates of unintended pregnancies in sub-Saharan Africa increase the prevalence of unsafe abortions in nations. The use of conventional steroids and non-steroid based contraceptives though effective is also linked to increasing side effects. The roots decoction of *Cissus rotundifolia* is used by women in Tana River County, Kenya as a fertility regulator. The study evaluated the phytochemical compounds present in the *Cissus rotundifolia* aqueous extract. It also evaluated the effect of the plant extract on oestrus cycle and other selected female reproductive parameters; mating success, fertility index, gestation length, and litter size using female Wistar rats. The phytochemical screening established the presence of alkaloids, tannins, saponins, phenols, and glycosides in the root aqueous extract of *Cissus rotundifolia.* The plant extract caused a dose-dependent significance increase in proestrus and metestrus phases and a significant reduction in estrus and diestrus phases ($P<0.05$). There was no significant difference in mating success. It caused a dose-dependent reduction in fertility index compared to the control. Gestation length was significantly increased and litter size significantly reduced ($P<0.05$). This probably is the reason for the traditional use of the plant as a fertility regulator. However, further work on reversibility, reproductive hormonal profile and ovarian histomorphology should be undertaken to improve the novel contraceptive pool.

Keywords: *Cissus rotundifolia*, oestrus cycle, phytochemical compound, mating success, fertility index, litter size, gestation length

*Correspondence to:
Anita Mary Mziray, Department of Biological Sciences, Egerton University, P.O. Box, 536-20115, Njoro, Kenya
anitamziray14@gmail.com

Cite This Article:
Mziray, A.M., Maina, C.I., Kaingu, C.K. 2020. Anti-fertility properties of *Cissus rotundifolia* (Forssk.) Vahl. Extract using female Wistar rats. *Discovery Phytomedicine* 7(2): 58-64. DOI: 10.15562/phytomedicine.2019.120

INTRODUCTION

Reproductive health is an important feature of socio-economic development. A global recent increase in contraceptive use has coincided with an increment of 25 million unintended pregnancies (Sedgh and Hussain, 2014). This might probably be due to inappropriate use, consistency challenges and possible method failures in fertility regulation. Fifty percent of youth aged below 25 years in Africa have inadequate access to reproductive health-care services (Lule et al., 2007). In Kenya, unmet contraceptive need is linked to a myriad of factors such as women's educational levels, location, autonomy, and income status among others (Campbell and Graham, 2006; Sedgh et al., 2007). The use of conventional steroids and non-steroid based contraceptives though effective is also linked to increasing side effects. It is therefore imperative to pursue alternative novel contraceptive options that are affordable, accessible, reversible and efficacious.

Cissus rotundifolia (Forssk.) Vahl. (Family *Vitaceae*) is commonly in Tana River county, Kenya, to regulate fertility (Kaingu et al., 2013). However, the role of *Cissus rotundifolia* as a potential anti-fertility agent is not clear and the literature is scanty. This study aimed to elucidate the anti-fertility properties of the plant extract on oestrus cyclicity, mating success, fertility index, gestation length and litter size in female Wistar rats.

MATERIALS AND METHODS

Plant collection and extract preparation

The root barks of the plant were collected from Garsen, Itsowe and Ngao sub-divisions of Tana River County, Kenya in February 2019. The roots were then transported to the Department of Veterinary Anatomy and Physiology, University of Nairobi. The roots were thoroughly washed using tap water to remove the soil. They were cut into small pieces using a knife, dried under shade for two weeks. The dried roots were then ground into fine powder using a Cunningham grinder (Artsan Manufacturing, Massachusetts, USA). Three hundred grams of the root powder were put in 3 liters of boiling distilled water for one hour. The mixture was thereafter, allowed to cool, filtered and lyophilised for 48 hours until the extract was completely dry. The plant aqueous extract yield was 205.45 grams. The extract was then kept in labelled sterile vials and stored in the refrigerator (-20°C) until use.

[1]Department of Biological Sciences, Egerton University, P.O. Box, 536-20115, Njoro, Kenya. cimainah@gmail.com
[2]Department of Veterinary Anatomy and Physiology, University of Nairobi, P.O Box 30197- 00100, Nairobi, Kenya. kaingucatherine@gmail.com

Experimental animals

Fifty mature normacyclic females aged 8-12 weeks were used in this study. They were purchased from the Biochemistry Department, University of Nairobi and kept in the animal house at the Department of Veterinary Anatomy and Physiology, University of Nairobi, Kenya. They were caged in groups of five and were maintained under standard environmental conditions of 12 hours light and 12 hours darkness at 24-25°C, and relative humidity of 30-70 percent. The rats were fed on diet pellets obtained from Unga Limited, Kenya and tap water provided *ad libitum*. Wood shavings were used as beddings and changed every other day to prevent the accumulation of urine and faecal waste. Their oestrus were monitored daily using vaginal smears for the first 10 days to ascertain regular cycle (Kaingu et al., 2017; Kaingu et al., 2018).

Only those with regular 4-5-day oestrus cycles were used for the experiment. Male Wistar rats were also kept in the same room but in different cages. Fertile male rats were introduced into female cages at the ratio of 1: 2 at the appropriate time (Kaingu et al., 2018). Animal Welfare and Ethics clearance was sought from the Faculty of Veterinary Medicine,University of Nairobi with reference number FVM BAUEC/2019/191.

Phytochemical Screening

A preliminary phytochemical screening of *Cissus rotundifolia* aqueous extract was carried out as per the methods described by Sahira and Catherine (2015) and Prashant et al., (2011) to decipher the presence of phytochemical compounds.

Effect of *Cissus rotundifolia* aqueous extract on oestrus cycle

The effect of *Cissus rotundifolia* aqueous extract on oestrus cycle was determined using fifteen nulliparous non-pregnant normal cyclic female Wistar rats. They were monitored daily for the first 10 days to ensure regular cyclicity. This was done by microscopical examination of vaginal smears to distinguish the four stages of the oestrus cycle (diestrus, proestrus, estrus, and metestrus).

The rats were divided into 3 groups of 5 rats each. Rats in Group I (control) received 0.5 ml of physiological saline, group II and group III received 400 and 800mg/kg of *Cissus rotundifolia* extract, respectively, through intra-abdominal lavage daily for 14 days. Vaginal smears were obtained from all the rats between 8 and 10 am and the oestrus cycle stages recorded (Kaingu et al., 2018). This was done by microscopical examination of vaginal smears to distinguish the four stages of the oestrus cycle (diestrus, proestrus, estrus, and metestrus).

Effect of *Cissus rotundifolia* aqueous extract on selected female reproduction parameters

The anti-fertility efficacy of *Cissus rotundifolia* aqueous extract on mating success, fertility index, gestation length, and litter size was evaluated using three treatment regimes on normocyclic female Wistar rats aged between 6-8 weeks. Male rats were introduced into female cages at the ratio of 1 male to 2 females at the appropriate time. Pregnancy was proven by taking vaginal smears daily and the first day of gestation was taken to be the day spermatozoa were detected in the vaginal smear under the light microscope.

Pre-mating extract administration

Fifteen rats were subdivided into three groups of five rats each. Sub-group A (control) received 0.5 ml of physiological saline. Sub- group B and C received 400 and 800mg/kg of *Cissus rotundifolia* aqueous extract respectively. These doses were administered daily for 14 days through intra-abdominal lavage. The rats were thereafter mated. Vaginal smears were still taken from the rats daily until pregnancy was proven (Kaingu et al., 2017).

The first day of gestation was taken to be the day spermatozoa were detected in the vaginal smear under the light microscope. This was noted as mating success.

Post-mating administration

Fifteen rats were divided into 3 subgroups (D, E and F) with 5 rats each. Water and pellets were provided *ad libitum*. All rats were mated. The first day of gestation was taken to be the day spermatozoa were detected in the vaginal smear under the light microscope.

Sub -group D (control) received 0.5 ml of physiological saline. Subgroup E and F received 400 and 800mg/kg *Cissus rotundifolia* aqueous extract respectively. These doses were administered daily through intra-abdominal lavage throughout the gestation length. The females were monitored daily by weighing until they littered (Kaingu et al., 2017). The litter size, as well as the gestation length of all rats, was noted.

Pre and post-mating administration

Fifteen rats were used. They were further divided into three subgroups with 5 rats each. Subgroup 1(control) received 0.5ml of physiological saline. Subgroup 2 and 3 received 400 and 800mg/kg *Cissus rotundifolia* aqueous extract respectively. The extract was administered for 14 days through intra-abdominal lavage after which the rats were

mated. Extract administration was continued after mating until litter (Kaingu et al., 2017).

Positive control

Positive control involved 5 normocyclic female rats being subcutaneously injected oestrogen/ progesterone(15µg oestradiol/ 0.15 mg progestin) once. The presence of spermatozoa in the vaginal smear was monitored daily to determine pregnancy.

Data analysis

Collected data was entered into MS- excel datasheet and sorted. Statistical analysis of the data was done using SPSS Statistical Software version 22. The data was tested for homogeneity of variance before the application of parametric tests. One- way ANOVA followed by post hoc test (Multiple comparison Dunnett t-test) was used to compare the outcomes between groups. All the results were expressed as mean ± standard error of the mean. P-values ($p<0.05$; $p< 0.01$) were considered significant.

RESULTS

Phytochemical composition of *Cissus rotundifolia*

Cissus rotundifolia aqueous extract showed the presence of five compounds -alkaloids, glycosides, phenols, saponins, and tannins. However, flavonoids and terpenoids were absent as shown in Table 1.

Effect of *Cissus rotundifolia* aqueous extract on oestrus cycle

400mg/kg of the aqueous extract of *Cissus rotundifolia* had a disruptive effect on the rats' oestrus cycle. Proestrus and metestrus phases increased to 4.00± 0.45 and 5.80±0.6, respectively. However, this increase was not significant ($p>0.05$) compared to the control group (3.40±0.66 and 3.20±0.20, respectively), over the 14-day extract administration period. (Figure 1). On the contrary, there was a significant decrease ($p<0.05$) in both the estrus and diestrus phases (2.80±0.58 and 1.20±0.37, respectively), compared to the control group (3.80±0.20 and 3.40±0.66, respectively), over the 14-day extract administration period.

Likewise, 800 mg/kg *Cissus rotundifolia* aqueous extract disrupted the oestrus cycle. There was a significant ($p<0.05$) increase in proestrus and metestrus phases (4.50±0.58 and 6.80±0.66, respectively), compared to the control (3.20±0.20 and 3.40±0.66, respectively). There was also a significant ($p<0.05$) decrease in both estrus and diestrus phases (2.00±0.71 and 1.80±0.45, respectively) compared to the control group (3.8±0.2 and

Table 1 **Phytochemical compounds present in the *Cissus rotundifolia* aqueous extract. (+)compound present; (-) compound absent**

Plant Extract	Alkaloids	Phenols	Flavonoids	Tannins	Saponins	Terpenoids	Glycosides
C.rotundifolia	+	+	-	+	+	-	+

Table 2 **Effect of *Cissus rotundifolia* aqueous extract on selected female reproduction parameters.**

	Mating success (%)	Fertility index (%)	Gestation length (days)	Litter size
Extract administration before mating (Pre-mating)				
Negative control	100	80	21.0 ±1.00	12.10 ±0.27
400mg/kg	100	80	23.8 ±0.21*	9.00 ±0.71*
800mg/kg	100	20 **	26.0 ±0.36*	5.00 ±0.04*
Extract administration after mating (Post-mating)				
Negative control	100	100	21.0 ±1.00	12.10 ±0.27
400mg/kg	100	60 *	24.0 ±0.12*	8.00 ±0.53*
800mg/kg	100	20 **	25.0 ±0.31*	4.00 ±0.20*
Extract administration before and after mating (Pre-post mating)				
Negative control	100	100	21.0 ±1.00	11.00 ±0.43
400mg/kg	100	20 *	26.0 ±0.11 *	5.00 ±0.36 *
800mg/kg	100	Nil	Nil	Nil
Positive control	Nil	Nil	Nil	Nil

Values of gestation length and litter size represent Mean ±SEM. Values with superscripts *, **, have a significant difference at ($p<0.05$; $p<0.01$) respectively compared to the negative control.

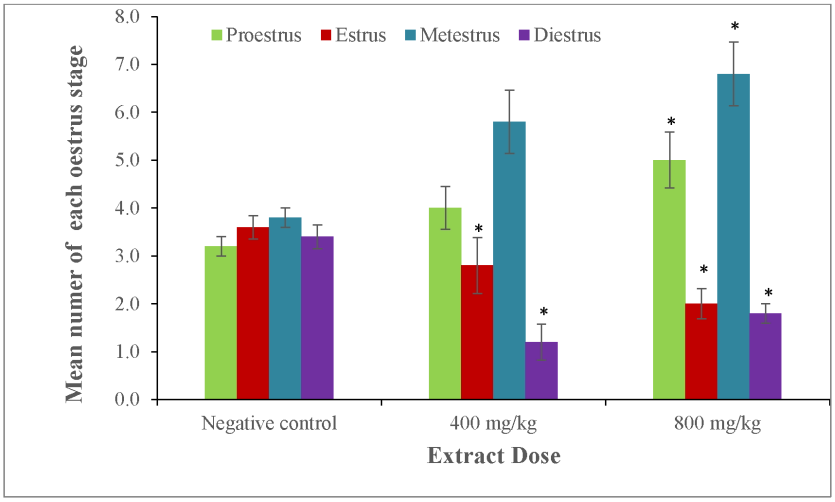

Figure 1 Effect of *Cissus rotundifolia* aqueous extract on oestrus cycle for a period of 14 days.Note: The vertical bars are ± SEM, * represents $p<0.05$

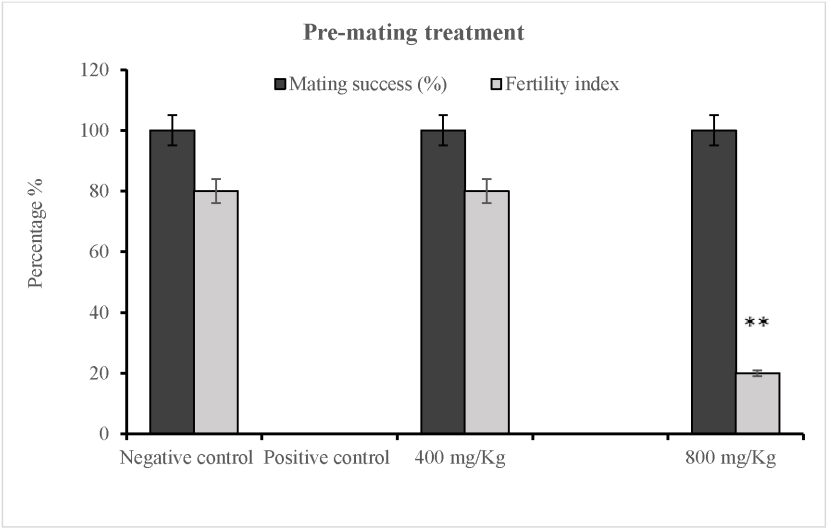

Figure 2 Effect of *Cissus rotundifolia* extract administered before mating on fertility index and mating success. Note: ** represents p<0.01

3.4±0.66, respectively) (Figure 1) over the 14 day treatment period.

The effect of *Cissus rotundifolia* aqueous extract on selected female reproduction parameters

Effect of Cissus rotundifolia extract administered before mating (pre-mating)

Cissus rotundifolia aqueous extract at both 400 and 800mg/kg doses had no significant effect (p>0.05) on mating success, compared to the control group. There was also no significant effect (p>0.05) of the extract at 400mg/kg dose on fertility index compared to the negative control. However, at 800mg/kg, the extract caused a significant reduction (p<0.01) in fertility index compared to the negative control (Table 2 and Figure 2). 400 and 800mg/kg caused a significant increase (p<0.05) in gestation length (23.8±0.21 and 26.0±0.36, respectively) compared to the control (21.0±1.00) (Table 4 and Figure 8). The litter size was significantly reduced (p<0.05) at both 400 and 800mg/kg (9.00±0.71 and 5.00±0.04, respectively), compared to negative control (12.10 ± 0.27).

Effect of Cissus rotundifolia extract administered after mating (Post-mating)

After mating regime (post-mating), both 400 and 800mg/kg *Cissus rotundifolia* extract had no significant effect (p>0.05) on mating success compared to control. At 400 mg/kg the extract caused a 60% significant reduction (P<0.05) in fertility index compared to 100% of the negative control.

800 mg/kg *Cissus rotundifolia* extract caused a significant reduction (p<0.01) in fertility index of 20% compared to 100% of negative control (Table 2 and Figure 3).

400 and 800 mg/kg *Cissus rotundifolia* extract caused a significant (P<0.05) increase of the gestation length to 24.0±0.12 and 25.0±0.31, respectively, compared to the control group (21 ± 1.00)

Cissus rotundifolia extract at 400 and 800mg/kg significantly increased (p<0.05) the gestation length to 24.0±0.12 and 25.0±0.31, respectively, compared to the control group (21±1.00).The litter size was also significantly reduced (p<0.05) at both 400 and 800mg/kg (8.00 ±0.53 and 4.00±0.20, respectively), compared to negative control (12.10 ± 0.27) (Table 2).

Effect of Cissus rotundifolia extract administered before and after mating (Pre-post mating)

400 and 800 mg/kg *Cissus rotundifolia* before and after mating regime (pre-post mating) caused no significant effect (p>0.05) on mating success compared to control. 400mg/kg extract caused a significant reduction (p<0.05) in fertility index to 20% compared to 100% of negative control. 800 mg/kg *Cissus rotundifolia* caused a significant reduction (p<0.05) in fertility index of 0% compared to 100% of negative control (Table 2 and Figure 4).

400 mg/kg *Cissus rotundifolia* extract caused a significant increase (P<0.05) of the gestation length to 26.0 ± 0.11 compared to the control group (21.0 ±1.00) (Table 2). At 800mg/kg, none of the rats littered because the fertility index was significantly reduced to 0%. The litter size was also significantly reduced (p<0.05) at 400mg/kg (5.00±0.36) compared to negative control (11.00±0.43). At 800mg/kg, none of the rats littered.

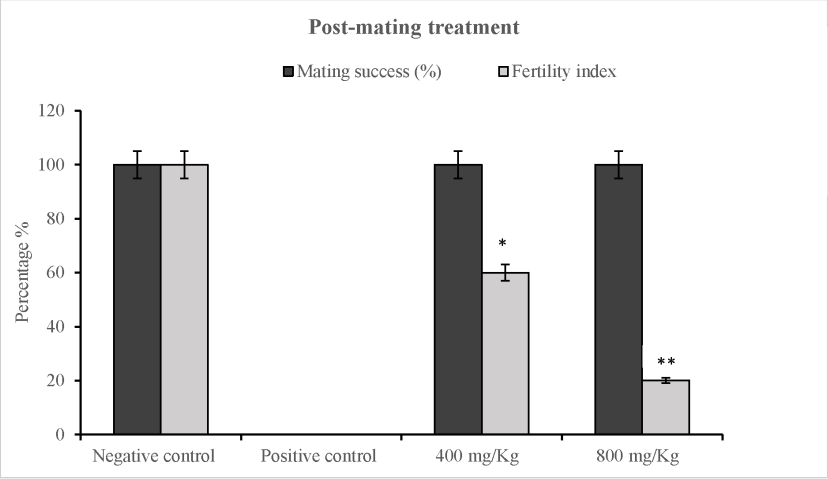

Figure 3 Effect of *Cissus rotundifolia* extract administered after mating on fertility index and mating success.Note: * represents p< 0.05, ** represents p<0.01

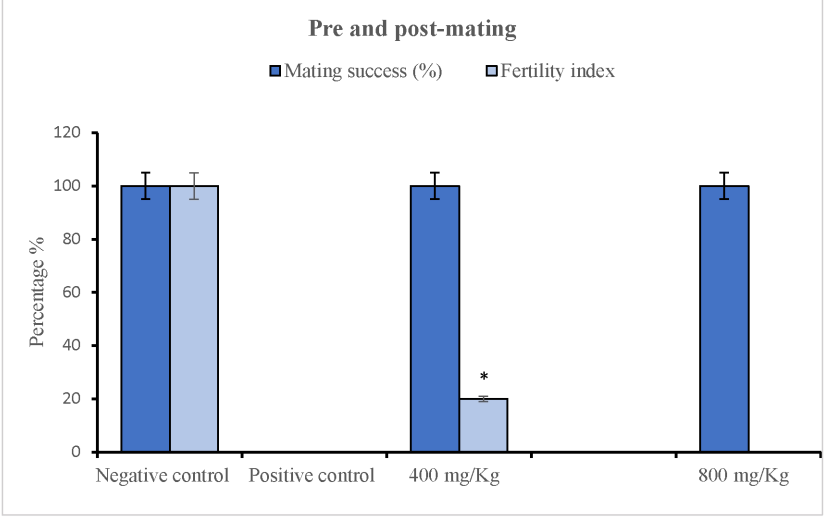

Figure 4 Effect of *Cissus rotundifolia* extract administered before and after mating on fertility index and mating success. Note *represents p<0.05

DISCUSSION

Phytochemical composition

Cissus rotundifolia roots are used by women in Tana River County, Kenya to control fertility. Preliminary phytochemical screening of the aqueous extract established the presence of alkaloids, phenols, tannins, saponins, and glycosides. However, flavonoids and terpenoids were absent (Table 1). From literature, phytochemical compounds are known to cause anti-fertility in females by acting as oestrus cycle disruptors, anti-oestrogenic agents, anti-implantation agents or abortifacient agents (Osonuga et al., 2014; Kurniati et al., 2017). Alkaloids inhibit ovulation, which subsequently disrupts oestrus cycle (Circosta et al., 2001; Sharma et al., 2014). Saponins, phenols, and tannins have an anti-implantation effect in rats (Ankush et al., 2011; Saravanan et al., 2012). Londonkar et al. (2009), working on crude *Sida acuta* extract, attributed its anti-fertility effect on rats due to the presence of phenols, tannins, and saponins which had anti-zygotic, blastocytotoxic and anti-implantation activity. Therefore, phytochemicals such as alkaloids, saponins, and phenols in the *Cissus rotundifolia* extract might be responsible for the anti-fertility effects of this plant. This validates the plant traditional use in Tana River County.

Oestrus cyclicity

Cissus rotundifolia root extract caused a significant increase in the frequency of proestrus and metestrus and a subsequent significant reduction in the frequency of estrus and diestrus phases at 800mg/kg. The hypothalamus and pituitary gland maintain progesterone and oestrogen balance as they play a critical role in ensuring normalcy in the histology and operation of the female genital tract (Pattanayak and Mazumdar, 2009; Namulindwa et al., 2015). In this study, *Cissus rotundifolia* might have interfered with hormone milieu resulting in the disrupted oestrus cycles. The findings of this study are consistent with those of Kaingu et al., (2018), who reported a reduction in estrus phase and a prolonged metestrus phase in female rats treated with aqueous extract of *Croton menyharthii* and *Uvariodendron kirkii*.

This study, however, is in contrast to that of Monsefi et al., (2015), who reported a significant increase in estrus and diestrus phases due to the effects of *Anethum graveolens* in female Wistar rats.

The oestrus cycle serves as a surrogate marker and has been frequently used to evaluate the impact of any anti-fertility agent (Kurnati et al., 2017). Thus, the disruption of the oestrus cycle due to the effect of *Cissus rotundifolia* extract makes the plant a potential anti-fertility agent.

Mating success, fertility index, gestation length and litter size

Mating was considered successful once a vaginal plug was established or the presence of spermatozoa in a vaginal smear was microscopically observed. In this study, mating success was 100% in all the treatment regimes at both 400 and 800mg/kg. This, therefore, indicates that ovulation occurred and the rats successfully mated. Fertility index was calculated as the number of pregnant rats divided by the total number of animals successfully mated multiplied by 100.

In the pre-mating treatment regime, 400 mg/kg *Cissus rotundifolia* caused no significant effect on fertility index and significant effect at 800 mg/kg (Table 2 and Figure 2). It is possible that fertilization

and/or implantation was disrupted by the high dose (800mg/kg) thereby leading to a reduction in fertility index. Fertility in females is determined by the developmental competence of an oocyte, the ability of the oocyte to be fertilized and give rise to a viable embryo, and for that embryo to successfully implant in the uterus. Interference of any of these processes could lead to fertility failure (Kaingu et al., 2018). This finding is similar to that of Dinesh et al. (2012), who reported a significant reduction in fertility index and no significant effect on mating success in female rats treated with *Bambusa vulgaris* aqueous leaf extract.

In the post-mating treatment regime, *Cissus rotumdifolia* caused a significant effect on the fertility index was significantly reduced for both doses (400 and 800mg/kg) (Table 2 and Figure 3). In this treatment, ovulation and fertilization had already occurred. This suggests that the post-mating extract administration either disrupted the implantation process or the uterine hormonal milieu that controls the process since endometrium receptivity is facilitated by ovarian steroids. However, gestation was still established probably because the ovarian steroids were optimal. This finding corroborates that of Azamthulla et al. (2015), who reported on anti-implantation effects of *Acalyphaindica indica, Ocimum sanctum* and *Butea monosperma* extracts in rats.

For the pre and post-mating extract treatment regime, *Cissus rotumdifolia* caused the most significant effect in fertility index at both doses (400 and 800mg/kg) (Table 2; Figure 4). This was probably due to its anti-ovulatory and/or anti-implantation properties. The plant extract probably disrupted the hypothalamus-pituitary-gonadal-axis thereby compromising gonadotropins release and ovarian steroids synthesis. These results corroborate those of Kaingu et al. (2018), who reported on a significant reduction in fertility index in female Wistar rats on a similar treatment regime using aqueous extract of *Croton menyharthii* and *Uvariodendron kirkii*.

Gestation length was significantly increased (Table 1) in all the three extract administration regimes probably due to the interference of oestradiol levels caused by the anti-estrogenic compounds, alkaloids and saponins present in the *Cissus rotundifolia* extract (Circosta et al., 2001; Sharma et al., 2014). Litter size was significantly reduced probably due to the effect of the plant extract on folliculogenesis and ovulation in Wistar rats.

CONCLUSION

Plants have the potential to be used as contraceptives and improve the contraceptive pool and so does *Cissus rotundifolia*. The presence of alkaloids, tannins, saponins, phenols, and glycosides might be responsible for the plant's anti-fertility activity. The disruption of the oestrus cycle and the effect of *Cissus rotundifolia* aqueous extract on reproductive parameters makes it a potential anti-fertility agent. However, further work on reversibility and effect on reproductive hormonal profiles and ovarian histomorphology should be undertaken.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

The authors acknowledge the Department of Biological Sciences, Egerton University and the Department of Veterinary Anatomy and Physiology, University of Nairobi, Kenya for their continued support until completion of this research work.

REFERENCES

1. Sedgh G, Hussain R. Reasons for contraceptive non-use among women having unmet need for contraception in developing countries. Stud Fam Plann. 2014; 5(2): 151-169.
2. Lule E, Singh S, Chowdhury S. Fertility Regulation Behaviors and Their Costs: Contraception and Unintended Pregnancies in Africa and Eastern Europe & Central Asia. Washington, DC: The International Bank for Reconstruction and Development / The World Bank. 2007.
3. Campbell O, Graham W.Strategies for Reducing Maternal Mortality: Getting on With What Works. Lancet. 2006 Oct 7; 368(9543): 1284-1299.
4. Sedgh G, Hussain R, Bankole A, Singh S. Women with an unmet need for contraception in developing countries and their reasons for not using a method. Occasional Report No. 37. June 2007.
5. Kaingu CK, Oduma JA, Mbaria JF, Kiama SG. Medicinal plants traditionally used for the management of female reproductive dysfunction in Tana River County, Kenya. Tang: International Journal of Genuine Traditional Medicine.2013 May 31; 3(2): 1-17.
6. Kaingu CK, Oduma JA, Mbaria JF, Kiama SG. Effects of *Croton menyharthii* and *Uvariodendron kirkii* extracts on ovarian corpora lutea and reproductive hormones. Discovery Phytomedicine 2017 April 20; 4(1): 21-25.
7. Kaingu CK, Oduma JA, Mbaria JF, Kiama SG. Anti-fertility and anti-implantation potential of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract in female Winstar rats. International Journal of Medicinal Plants. 2018 Jan 30; 112(2018): 844-855.
8. Sahira B, Catherine L. General techniques involved in phytochemical analysis. International Journal of Advanced Research in Chemical Science (IJARCS).2015 April; 2(4): 25-32.
9. Prashant T, Bimlesh K, Mandeep K, Gurpreet K. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia. 2011 Jan -March; 1(1): 98-106.
10. Osonuga OA, Osonuga IO, Osonuga A. Oral administration of leaf extracts of *Mormodica charantia* effects on reproductive hormones of adult Wistar rats. Asian Pac J Trop Biomed.2014; 4(1): S521-S524.
11. Kurniati R, Dewi H, Hendra M. Effect of water decoction of Langsat Bark (*Lansium domesticum Corr.*) on estrous cycle and uterus weight in mice (*Musmusculus L.*). The 7th International Conference on Global Resource Conservation,44. AIP Publishing. 2017.

12. Circosta C, Sanogo R, Occhiuto F. Effects of *Calotropis procera* on estrous cycle and on estrogenic functionality in rats. Farmaco Sci. 2001 July 1; 56(7): 373-378.
13. Sharma RK, Goyal AK, Bhat RA. Anti-fertility activity of plants extracts on female reproduction: A Review. Int J Pharm Biol Sci.2014 Jun-Sep; 3(3): 493-514.
14. Ankush R., Singh A, Sharma A, Bhatia V. Antifertility activity of medicinal plants on reproductive system of female rats. J Med Bioeng. 2011; 2(3): 44-50.
15. Saravanan K, Priya G, Renuka C. Medicinal plants with potential antifertility activity-A Review of sixteen years of herbal medicine research (1994-2010). Int J Pharmtech Res. 2012; 4 (1): 481-494.
16. Londonkar RL, Sharangouda JP, Saraswati BP. Phytochemical and contraceptive property of *Sida acuta burm*. in Albino rats. Int J Pharmtech Res. 2009; 1(4): 1260-1266.
17. Pattanayak SP, Mazumdar PM. Effect of *Dendrophthoe falcate* on the female reproductive system of Wistar rats. Contraception. 2009 April 23; 80(3): 314-320.
18. Namulindwa A, Nkwangu D, Oloro J. Determination of the abortifacient activity of the aqueous extract of *Phytolacca dodecandra* (L'Her) leaf in Wistar rats. Afr J Pharm Pharmacol. 2015 Jan 22; 9(3): 43-47.
19. Monsefi M, Mojaba M, Elham H, Farnaz G, Ramin M. Anti-fertility effects of different fractions of *Anethum graveolens L*. extracts on Oocytes and Fertility on adult female rats. J Reprod Infertility. 2015 Jan -Mar; 6(1): 10-17.
20. Dinesh K, Ajay K, Prakash M. Potential anti-fertility agents from plants: A comprehensive review. J Ethnopharmacol.2012; 10(55): 1-32.
21. Azamthulla M, Rajkapoor B, Kavimani S. A Review on medicinal plants exhibiting antifertility activity. World J Pharm Pharm Sci.2015; 4(3): 243-272.