World Journal of Innovative Research (WJIR)
ISSN: 2454-8236, Volume-10, Issue-4, April 2021 Pages 156-159

Hydroethanol Leaf Extract of Cissus Aralioidesalters Oestrous Cycle and Reproductive Hormones in Female Experimental Wistar Rats

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***Abstract*— Infertility has been a major concern among couples globally, especially in Africawhere herbal therapy is common, without proper assessment of their adverse effects. This study aims at evaluating the effects of Cissus aralioidesreproductive functions.25 adult, regular cycling female wistar rats were divided into 4 groups of 5 rats each; group1(control) receivedwater, treatment groups 2, 3 and 4 received 150, 300 and 600 mg/kg bw of hydroethanol leaf extract of C. aralioides respectively orally for 25 days. oestrus cycle was evaluated daily. On day 26, blood sample was collected for hormonal assay, and ovaries for tissue biochemicalanalysis.Result showed significant (P<0.05)increase incycle length and decreased cycle frequency during the study; significant (P<0.05) decrease inFSH, LH and Estradiol; increased Progesterone, TP, TC, SOD,CATand MDA. We conclude that the extract alters ovarian functions by reducing FSH, LH and estradiol while increasing progesterone, ovarian cholesterol and protein, and therefore alters oestrous cyclicity in rats.**

***Index Terms*— Cissus aralioides,Oestrus cycle, Reproductive hormones, ovarian functions, infertility.**

I. INTRODUCTION

The *Cissus aralioides* (Welw ex. Barker) plant belongs to the family *Vitaceae* and is predominantly cultivated in tropical Africa.It is found indeciduous forests and fringing jungles across Senegal to Northern and Southern Nigeria (Burkill, 2000, Ezeja et al., 2015a). The plant is commonly known as monkey plum (Adelanwa and Haruna, 2013).Phytochemical component of the plant include vitamins, terpenoid, phenolic acids, lignins, stilbenes, tannins, flavonoids, saponins, steroids, terpenes, cardiac glycosides, quinones, protein, reducing sugar, coumarins, alkaloids, amines, betalinins, and other metabolites.(Borokini and Omotayo 2012)

Reports suggest the potential of *Cissus aralioides* plant to influence endocrine activities in both humans and animals due to its numerous chemical constituents (Drewes et al., 2003; Cao et al., 2008). One of the most recent works on the plant is that of Nwogueze et al. (2018) which investigated the toxicity of aqueous extract of *Cissus aralioides* plant. The results of the acute toxicity showed LD_{50} greater than 5000 mg/kg body weight. Aigbiremolen *et al* (2018)showed that the aqueous leaf extract of *Cissus aralioides* plantdecreases body weights of rats and may impair other biochemical activities. Given that that this plant is widely consumed for its numerous ethnomedicinal values, and the increasing gobal concern of increasing incidence of reproductive dysfunction, this study was designed to assess the effect of *Cissus aralioides* leaf extract on reproductive functions in in females using *wistar* rats as experimental model.

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II. MATERIALS AND METHODS

Plant Collection and preparation of Extrat The *Cissus aralioides*leaves used for this study was sourced and collected from a local forest in Etche Local Government Area of Rivers state, and was identified and authenticated at the Plant Science and Biotechnology Department of the University of Port Harcourt with Herbarium Number (UPH/P/185). The dried leaves were ground into powder and 3 kg of the powder was mixed with 6000 mL of hydro-ethanol (30:70) in a maceration jar. The extraction was doneby soxlet extraction method.

Experimental Animals

Adult female *wistar* rats weighing 160-200g were used. The animals were allowed to acclimatize for 14 days and were maintained in the faculty of Basic Medical Sciences animal house, under standard environmental conditions:temperature (25 ± 2^0C), relative humidity (50 ± 10%), andnatural light/dark cycles throughout the experimental period. The animals had free access to water and feed (Standard pellet diet)*ad libitum*. The experimental protocols were approved by University of Port HarcourtResearch and Ethics Committee with reference: (UPH/CEREMAD/REC/MM72/006).

Experimental Design

A total of 25 adult female wistar rats with regular oestrous cycles were randomly divided into 4 groups of 5 animals each.Group 1 (control) received 1ml distilled water, whiletest groups 2, 3 and 4received 150, 300 and 600 mg/kg bwrespectively of the hydroethanol leaf extract of *Cissus aralioides*. The administration of the extract was once daily by oral gavage for 25 days.During the treatment period, the oestrous cycle of the animals were dailyevaluated according tostandard method(Goldman et al., 2007). At the end of the 25 day administration period, the animals were sacrificed under light chloroform anaesthesia. The ovaries of the animals were harvested, weighed and fixed in Bouins fluid

for determination of ovarian histology and tissue biochemical parameters according to the method of Rukmini et al. (2004) and Ahangarpour et al. (2015).Blood (3ml) samples were collected by cardiac puncture for analysis of reproductive hormones (Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Progesterone(P) and Estradiol (E)) by Enzyme linked immunoassay method as earlier reported(Ahangarpour et al.,2015).

Statistical Analysis

The statistical analysis of data was done for one factor analysis of variance (ANOVA) followed by post hoc analysis using Statistical Package for Social Science (SPSS) version 25. The resulting data were presented in tables while the continuous variables were presented as mean and standard error of mean (mean ± SEM) and differences considered significant at P< 0.05.

III. RESULTS

Table 1 shows effect of the extract on the length and number of oestrous cycles during the treatment period (25 days). There was a dose dependent increase in the oestrous cycle length for animals treated with 150mg/kg (P>0.05), 300mg/kg (P<0.05) and 600mg/kg (P<0.05) bw respectively, compared to the control group. On the other hand, the number of oestrous cycles was reduced in all the extract treated groups, with significant decrease observed in the 300mg/kg and 600mg/kg groups, compared to the control.

Table 1: Effect of hydroethanol leaf extract of *C.aralioides* on Oestrous cycle.

Groups	Length of Oestrous Cycle (days)	Number ofCycles (In 25 days)
Control	4.52 ± 0.13	6.20 ± 0.37
150 mg/kg Extract	4.88 ± 0.09	6.00 ± 0.31
300 mg/kg Extract	8.10 ± 0.24*	2.60 ± 0.40 [a]
600 mg/kg	7.71 ± 0.32*	2.80 ± 0.58 [a]

Data are expressed as mean ± SEM, n=5

*= significantly different compared to control (P<0.05)

Table 2 shows he effect of the extract on gonadotropic hormones(folicle stimulating hormone and leuteinising hormone) and the female sex hormones (Progesterone and Estradiol). Follicle stimulating hormone (FSH) significantly (P<05) decreased in all the extract treated groups, compared to the control, while Leuteinising hormone (LH) level also decreased in all the extract treated groups, with a significant (P<0.05) decrease observed only in the 150mg/kg and 600mg/kg bw groups respectively, compared to the control group.

Additionally, a significant (P<0.05) dose-dependent increase in serum progesterone was observed in all the extract treated groups, compared to the control group. Estradiol, on the other hand, did not show any significant difference in the test groups, when compared to the control group, except in the highest dose of extract (600mg/kg) where there was a significant (P<0.05) reduction.

Table 2: Effect of *Cissus aralioides*leaf extracton gonadotropic and female rsex hormones.

Groups	FSH (mIU/L)	LH (mIU/L)	Progesterone (ng/ml)	Estradiol (pg/ml)
Control	35.34 ± 9.95	16.23 ± 2.78	2.87 ± 0.94	5.12 ± 1.59
150 mg/kg	*9.25 ±0.85	*7.18 ±1.04	*11.06 ± 1.21	5.08 ± 2.28
300 mg/kg	*14.99 ± 1.45	14.73 ±1.47	*15.88 ± 2.23	6.82 ± 4.07
600 mg/kg	*7.55 ± 0.42	*9.92 ±0.17	*16.98 ± 0.56	*3.51 ± 0.73

Data are expressed as mean ± SEM, n=5, *= significantlydifferent from control group (P<0.05)

As shown in Table 3, administration of the extract significantly (P<0.05) increased the level of catalase (CAT) and superoxide dismutase (SOD) enzymes activities, in the extract treated groups, except the lowest test group (150 mg/kg) where the difference in SOD was not significant (P>0.05), when compared to their respective control groups.

Also, a significant (P<0.05) change was noticed in the serum level of malondialdehyde (MDA),where the animals treated with 150 mg/kg and 600 mg/kg extract showed significant (P<0.05) increase, while the 300 mg/kg group was seen to be significantly (P<0.05) lower,compared to the control groups.

Furthermore, the level of Total Protein and total Cholesterol(Table 4) were significantly (P<0.05) increased in all the extract treated test groups (150 mg/kg, 300mg/kg and 600 mg/kg), when compared with the control.

Table 3: Effect of *Cissus aralioides* leaf extract on Ovarian Tissue oxidative stress markers.

Groups	CAT (µ/mL)	SOD (µ/mL)	MDA (µ/mL)
Control	65.10 ± 10.14	39.06 ± 9.34	53.77 ± 3.50
150 mg/kg	*189.40 ± 41.35	37.26 ± 5.82	*66.93 ± 3.59
300 mg/kg	*307.78 ± 13.05	*72.96 ± 5.14	*42.04 ± 0.99
600 mg/kg	*230.74 ± 28.87	*120.91 ± 7.21	*65.30 ± 1.13

Data are expressed as mean ± SEM, n=5, *= significantly different from control group (P<0.05)

Table 4: Effect of hydroethanol leaf extract of *C. aralioides* on Ovarian tissue biochemical parameters

Groups	Total Protein (g/L)	Total Cholesterol (mmol/L)
Control	42.62 ± 0.59	4.08 ± 0.62
150 mg/kg	*48.26 ± 1.06	4.36 ± 0.43 [a]
300 mg/kg	*47.19 ± 0.36	4.53 ± 0.74 [a]
600 mg/kg	*48.649 ± 0.51	4.56 ± 0.32 [a]

Data are expressed as mean ± SEM, n=5
a – significantly different from control group (P<0.05)

IV. DISCUSSION AND CONCLUSION

This study has demonstrated that administration of 300 and 600mg/kg BW of *C. aralioides*leaf extract significantly increased the length of the oestrus cycles, thereby reducing the frequency of the cyclesduring the treatment period. Similar results have been obtained with other plant extracts such as *Achyranthes aspera* (Shibeshi et al., 2006), *Trichosanthes cucumerina L. var. Cucumerina* (Kage *et al.*, 2009). Prolongation of the oestrus cycle following administration of the extract suggests alterations in the activities of the hypothaamo - pituitary - ovarian axis. This may be due to the presence of saponins and alkaloids in the extract (Lemuel et al., 2019).

These alterations are also demonstrated by the observed reductions in the pituitary secretion of follicle stimulating hormone and leuteinising hormone levels. This decrease in pituitary gonadotropins indicates that the extract may have acted on the anterior pituitary gland to impair or inhibit the secretion of these gonadotropin hormones (Priya et al., 2004). this agrees with Yakubu *et al*. (2008) which reported that the aqueous extract of *C. aralioides* reduced the serum concentrations of FSH and LH. This could result to a possible decline of ovarian functions as both FSH and LH are required for folliculogenesis and ovulation (Kage et al., 2009).

The extract also altered the level of the female sex hormones. The increase in progesterone level shows that the neuroendocrine regulation of the graafian follice annd corpus luteum was altered resulting in sustained secretion of progesterone, which caused the prolongation of the oestrous cycle observed in this study. This study also show that at high dose (600mg/kg) of the extract, there was reduction in oestradiol level, similar to report of Nwogueze et al. (2019), who demonstrated a dose-dependent decrease in estrogen level in animals administered with 300mg/kg and 500mg/kg BW of aqueous leaf extract of *C. aralioides*. The reduction in oestradiol suggests impaired growth and maturation of the follicles, under the influence of FSH, as matured follicles secrete oestradiol. Therefore, the reduction in FSH impaired the growth and maturation of the follicles with resultant decrease in the secretion of oestradiol. This agrees with the assertion of Amah *et al.,* (2012) and Ladan *et al.,* (2013) which state that suppressing the release of the pituitary gonadotropins could ultimately result in alterations in the oestrous cycle as well as the interaction and secretion of sex hormones

The plant *Cissus. aralioides* contains many bioactive as well as toxic agents such as saponins, flavonoids and alkaloids (Yakubu et al., 2008; and Nwogueze *et al.* 2018), which possess anti-fertility activity that can affect the hormonal regulation of oestrous cycle, conception and reproduction (Okon and Etim, 2014), as well as reduce plasma concentrations of LH, estradiol, progesterone and FSH (Bianco, Basini & Grasselli, 2006; Yakubu et al., 2008).Therefore, the alterations in the oestrous cycle, and the levels of the circulating hormones observed in this study may be attributed to the presence of these phytochemicals.

This study also analyzed the level ovarian tissue biomarkers inorder to evaluate the influence of oxidative stress in the ovarian tissue on the observed effects of the extract. We observed that both the antioxidant enzymes (CATand SOD)and MDA, as well as total protein and total cholesterol increased in the testicular tissues.

Increase in MDA is an indicator of lipid peroxidation (Adienbo et al., 2015),leading to enhanced level of reactive oxygen species (ROS) in the tissue resulting in oxidative stress and tissue damage (Khaki, 2009; Soltani et al., 2018; Ameli et al., 2018).

Also, cholesterol is the principle starting key molecule required for steroid hormone biosynthesis within the ovary

(Kage *et al.*, 2009), while proteinsare required in the metabolic activities in the ovaries. Hence, their increase in the ovaries as seen in this study shows poor utilisation and impaired metabolic and secretory functions of the ovaries,and an indication of the deprivation of tropic support by the pituitary gonadotropins.

V. CONCLUSION

In conclusion, this study has demonstrated that the hydroethanol leaf extract of C. *aralioides* prolongs oestrous cycle length,impairs pituitary secretion of FSH and LH, and alters progesterone and oestradiol levels in rat, through disruption of ovarian utilisation of cholesterol and proteins. These effects may be due to the saponins, flavonoids and alkaloids present in the *extract.*

REFERENCES

[1] Adelanwa, M. A., and Haruna, H. B. (2013). Survey of some plants found in gurara local government area of Niger state, Nigeria: *InternationalJournal of Applied Biological Research*, *5*(1), 43 – 54

[2] Adienbo, O. M., Nwafor, A., and Dapper, D. V. (2015). Impairments in testicular function indices in male wistar rats: a possible mechanism for infertility induction by *Xylopia aethiopica* fruit extract. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, *4*(1), 71 - 75

[3] Ahangarpour, A., Lamoochi, Z., Moghaddam, H. F., Mansouri, S. M. T. (2015). Effects of Portulaca oleracea ethanolic extract on reproductive system of aging female mice. *International Journal of Reproductive BioMedicine*, *14*(3), 205 – 212

[4] Aigbiremolen, A. A., Ihegihu, E. Y., & Udi, O. A. (2018). Morpho-functional Changes in Reproductive Statusof Female Wistar Rats Fed with *Cissus aralioides*Aqueous Extract. *International Journal of Research and Reports inGynaecology*, *1*(1), 1 – 9.

[5] Amah, C. I., Yama, O. E., & Noronha, C. C. (2012). Infecund evaluation of cycling female Sprague-Dawley rats: an aftermath treatment with *Momordicacharantia* seed extract. *MiddleEast Fertility Society Journal*, *17*(1), 37 – 41.

[6] Ameli, M., Hashemi, M. S., Moghimian, M., & Shokoohi, M. (2018). Protective effect of tadalafil and verapamil on testicular function and oxidative stress after torsion/detorsion in adult male rat. *Andrologia*, *50*(8). doi:10.1111/ and.13068

[7] Bianco, F., Basini, G., & Grasselli, F. (2006). The plant alkaloid *Sanguinarine* affects swine granulose cell activity. *Reprod Toxicol.*, *21*, 335 – 340.

[8] Benie, T., Duval, J., & Thieulant, M. L. (2003). Effects of some traditional plant extracts on rat oestrous cycle compared with clomid. *Phytother Res.*, *17*, 748 – 755.

[9] Borokini, T. I., & Omotayo, F. O. (2012). Comparative phytochemical analysis of selected medicinal plants in Nigeria. *International Journal of Advanced Chemical Research*, *1*(1): 11 – 18.

[10] Burkill, H. M. (2002). The useful plants of West Tropical Africa, Families. *Royal Botanical Gardens Kew*, *5*, 301 – 302.

[11] Cao, D. P., Zheng, Y. N., Qin, L. P., Hana, T., Zhang, H., Rahman, K., & Zhang. Q.Y., (2008). Curculigo orchioides, a traditional Chinese medicinal plant, prevents bone loss in ovariectomized rats. *Maturitas*, *59*, 373 – 80

[12] Drewes, S. E., George, J., & Khan, F. (2003). Recent findings on natural products with erectile dysfunction activity. *Phytochemistry*, *62*: 1019 – 1025.

[13] Edeoga. H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigeria medicinal plants. *African Journal of Biotehnology*, *4*(7), 685 – 688.

[14] Ezeja, M. I., Omeh, Y. N., Onoj, S. O., & Ukaonu, I. H. (2015a). Anti-inflammatory and Antioxidant Activities of the Methanolic Leaf Extract of *Cissus aralioides*. *American Journal of Pharmacology Science*,*3*(1), 1 – 6.

[15] Ezeja, M. I., Onoja, S. O., Ukwueze, C. O., & Madubuike, G. K. (2015b). Evaluation of the analgesic activity of *Cissus aralioides* leaves in rat. *Journal of Chemical and Pharmaceutical Research*, *7*(3), 2525 – 2528.

[16] Goldman, J. M. (2007). The rodent estrous cycle: characterizationof vaginal cytology and its utility in toxicologicalstudies. reproductive toxicology, 80, 84-87.

[17] Kage, D. N., Malashetty, V. B. Seetharam, Y. N., Suresh, P., & Patil, S. B. (2009). Effect of ethanol extract of whole plant of *Trichosanthes cucumerina* var. *Cucumerina* L. on gonadotropins, ovarian follicular kinetics and estrous cycle for screening o antifertility activity in albin rats. *Int. J. Morphol.*, *27*(1), 173 – 182.

[18] Khaki, A. (2009). Evaluation effects of quercetin on liver apoptosis in streptozotocin-induced diabetic rat. *Journal of Medicinal Plant*, *1*(29), 70 – 78

[19] Ladan, K., Elmira, H., Arefeh, M., & Shahin, A. (2013). Knowledge, attitude and practice of herbal remedies in a group of infertile couples. *ActaMedicaIranica*,*51*(3), 189 – 194.

[20] Lemuel A. M, Muhammad B, Sodiq L, Echoru I, Ssempijja F, Owembabazi E… (2019). Effect of cleome gynandra leaf extract on the estrous cycle and histology of the ovary and uterus of wistar albino rats. Anatomy Journal of Africa.Vol 8 (1): 1385 -1394

[21] Noguchi, E., Fujiwara, Y., Matsushita, S., Ikeda, T., Ono, M., & Nohara, T. (2006). Metabolism of tomato steroidal glycosides in humans. *Chem. Pharm. Bull.*, *54*, 1312 – 1314.

[22] Nwogueze, B. C., Anachuna, K. K., Eke, C. N., & Ufearo, C. S. (2018). Toxicity Studies and Phytochemical Screening of Aqueous Extract of *Cissus aralioides* Plant. *Journal of Advances in Medical and PharmaceuticalSciences*, *17*(1), 1 – 7

[23] Nwogueze, B. C., Ojieh, A. E., Ossai, R. N., Eke, C. N., & Ufearo, S. C. (2019). Reproductive Function Evaluation in Female Wistar Rats Treated with Aqueous leaf Extract of *Cissus aralioides. Biosciences Biotechnology Research Asia*, *16*(4), 857 – 864

[24] Okon, U. A., & Etim, B. N. (2014). *Citrus aurantifolia* impairs fertility facilitators and indices in male albino wistar rats. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, *3*(3), 640 – 645.

[25] Priya, P. N., Pillai, A., & Gupta, S. (2004). Effect of Simultaneous Exposure to Lead and Cadmium on Gonadotropin Binding and Steroidogenesis on Granulosa Cells: An in vitro Study. *Indian J Exp Biol*, *42*, 143 – 8

[26] Rukmini, M. S., D'souza, B., D'souza, V. (2004). Superoxide dismutase and catalase activities and their correlation with malondialdehyde in schizophrenic patients. *Ind. J. Clin. Biochem.*, *19*(2), 114.

[27] Shibeshi Workeneh, Eyasu Makonnen, Asfaw Debella, Legesse Zerihem(2006). Phytochemical, contraceptive efficacy and safety evaluation of the methanolic leaves extract of Achyranthes Aspera L. in rats.

[28] Soltani, M., Moghimian, M., Abtahi-Eivari, S. H., Shoorei, H., Khaki, A., & Shokoohi, M. (2018). Protective effects of *Matricaria chamomilla* extract on Torsion/ detorsion-induced tissue damage and oxidative stress in adult rat testis. *International Journal of Fertility and Sterility*, *12*(3):242 – 248.

[29] Uchewa, O., & Ezugworie, J. O. (2018). The efficacy of herbal products as antioxidant in cushioning the effects of short-term exposure of female rats to low dose of environmental toxicity. *Pharmaceutical Bioprocessing*, *6*(4), 142 – 153

[30] Yakubu, M. T., Akanji, M. A., Oladiji, A. T., Olatinwo, A. O., Adesokan, A. A., Yakubu, M. O., Owoyele, B. V.,Sunmonu, T. O., & Ajao, M. S. (2008).Effect of *Cnidoscolous aconitifolius*(Miller) I.M.Johnston leaf extract on reproductive hormones offemale rats.*Iranian Journal of Reproductive Medicine*, *6*(3), 149 – 155