EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 9
217-221
Year: 2021

ASSESSMENT OF ACTIONS OF *CATHARANTHUS ROSEUS* ON PREGNANCY IN WISTAR RATS

P. E. Dabor[1*], C. Obiandu[2] and H. O. Asuzu-Samuel[3]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria.
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.
[3]Department of Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt, Choba, Rivers State, Nigeria.

***Corresponding Author: P. E. Dabor**
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria.

Article Received on 15/07/2021 **Article Revised on 04/08/2021** **Article Accepted on 24/08/2021**

ABSTRACT

Introduction: The use of *Catharanthus roseus* in the treatment of some illnesses in traditional medicine practice is common. Its use in the treatment of co-existing conditions in pregnancy is common but its effects on pregnancy is largely controversial. **Aim:** The aim of this study is to evaluate the effect of methanol leaf extract of *Catharanthus roseus* on some reproductive parameters, weight of fetus and pregnancy outcome in female wistar rats. **Method:** Twenty five (25) pregnant wistar rats were used in this study. They were initially cohabited with males until pregnancy was achieved. Animals were divided into five (5) groups. Group 1 served as control. Groups 2, 3, 4 and 5 were orally administered (125, 250,500 and 1000) mg/kg body weight of *Catharanthus roseus* extract respectively, from day 7th to 16th of pregnancy. Animals were sacrificed on 17th day of pregnancy. Blood was collected by cardiac puncture for hormone analysis. **Results:** The results revealed that the serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and estrogen were significantly ($P<0.05$) decreased compared to control group. However, a significant increase in prolactin level was observed. Furthermore, there was a significant ($P<0.05$) loss in maternal body weight. A similar reduction also occurred in the weight of fetus. A significant increase in percentage of dead fetus was observed in animals that took higher doses (up to 500mg/kg) of the extract. **Conclusion:** This study showed that methanol leaf extract of *Catharanthus roseus* may possess abortifacient potentials and may increase fetal loss.

KEYWORDS: *Catharanthus roseus*, pregnant wistar rats, abortifacient, methanol.

1.0 INTRODUCTION

Catharanthus roseus is a medicinal plant with a global spread but predominantly found in the East Island of Madagascar. It is a perennial shrub with a woody base growing up to 1 meter in height. It produces an unpleasant smell with white latex.[1] It has been identified using various names such as old maid, rose periwinkle, cape periwinkle and simply as periwinkle plant.[2] The use by traditional healers in curing people has spanned over decades. The plant has been used in traditional remedies for muscle pain, bleeding nose and bleeding gums, mouth ulcers, memory loss, hypertension, diarrhoea and hyperglycemia.[3] Some studies carried out showed that extracts of *Catharanthus roseus* possess antimicrobial,[4] antioxidant,[5] antihelmintic,[6] antidiarrheal as well as antidiabetic properties.[7,8] A chemist in the 1950s who searched for biologically active compounds in *Catharanthus roseus* revealed that the plant also possessed anti-cancer properties when he showed the presence of the compounds, vincristine and vinblastine which has been employed as drugs for treating diseases, especially cancer since the early 1960s.[9] The alkaloids and phenolics were also reported to be very important bioactive compounds of *Catharanthus roseus*.[10] These bioactive compounds are reportedly responsible for causing many of the pharmacological actions of *Catharanthus roseus*.

One of the disturbing observation among users of medicinal plant products for therapeutic purposes is self medication. The practice of self-medication among the populace including pregnant women in the rural or local community is common especially, in Africa.[11] These pregnant women take these medicinal plant preparations to treat some health conditions such as increased blood

sugar level (gestational diabetes) without any knowledge of the effects these products may have on the pregnancy.[12]

There is paucity of scientific data on the effects of use of *Catharanthus roseus* in pregnancy. The aim of this study is to assess the actions of methanol leaf extract of *Catharanthus roseus* on some reproductive parameters, weight of fetus and pregnancy outcome in female wistar rats.

2.0 MATERIALS AND METHODS

2.1 Collection and extraction of plant material

The fresh leaves of *Catharanthus roseus* (Periwinkle plant) were collected from the botanical garden of Imo State University (IMSU), Owerri, Nigeria. The plant was identified by a taxonomist at the herbarium unit of the Department of Plant Science and Biotechnology at the University of Port Harcourt with herbarium number UPH/P/255. An approval for this study was obtained from the centre for Research ethics and management of the University of Port Harcourt, Nigeria with certificate number UPH/CEREMAD/REC/MM77/012.

The fresh leaves of *Catharanthus roseus* was dried for two weeks. The dried samples were ground into powder form using a grinding machine.

A total of 500g of the leaves was obtained after grinding and the powdered leaves sample was dissolved in 400ml of methanol for 72 hours with constant stirring to enable the leaves to dissolve in the solvent, the solvent used is 100% methanol. The solution was filtered using a filter paper. The filtration was repeated three times to achieve a clear filtrate. The filtrate was then transferred into a rotary evaporator to remove the methanol from extract by means of concentration. The semi-liquid substance was poured into an evaporating dish and placed on a stem bath for complete drying at a temperature of 45°C to 50°C. The drying was monitored until it turned into a paste form. The percentage yield of *Catharanthus roseus* was 63.1g. The extracts were moved to air-tight vials and maintained at 4°C, away from light before use for the study.

2.2 Study area

This experimental study was carried out in the Department of Human Physiology, Faculty of Basic Medical Science in University of Port Harcourt, Nigeria.

2.3 Experimental animals and protocols

Twenty five (25) mature, post pubertal female wistar rats were used in this study. They were bred in the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria, and were housed in standard rat cages. The animals were allowed 14 days to acclimatize and were weighed using a digital scale with accuracy of 0.001 gram. They had access to water and feeds *ad libitum.* The female wistar rats estrous cycle was checked to give a guide in the grouping of the female rats according to their different phases of estrous. They were initially cohabited with males until pregnancy was achieved. Confirmation of mating and vaginal plug of each female was recorded as first day of pregnancy. Experiments carried out were in agreement with the National Institutes of Health's Guide for the care and use of laboratory animals.[13]

2.4 Experimental Design

Animals were divided into five (5) groups. Group 1 served as control and received distilled water. Groups 2, 3, 4 and 5 were orally administered (125, 250,500 and 1000) mg/kg body weight of *Catharanthus roseus* leaf extract respectively, from day 7^{th} to 16^{th} of pregnancy. The entire administration was by oral gavage once daily. Animals were sacrificed on 17^{th} day of pregnancy. All animals were anesthetized using 25% urethane i.p. Blood was collected by cardiac puncture into lithium heparin bottles for estimation of some female reproductive hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH) progesterone, estrogen and prolactin. The fetuses were collected and weighed. The lethal dose used was 5000mg/kg bw as previously reported.[14]

2.5 Statistical Analysis

This was achieved by the application of Statistical Package for Social Science (SPSS) version 23. One-way ANOVA was employed to analyze and compare mean of the various groups and results expressed as Mean ± standard error of mean. Values of $P < 0.05$ were considered significant.

3.0 RESULTS AND DISCUSSION

The present study assessed the actions of methanol leaf extract of *Catharanthus roseus* on some reproductive hormones, weight of fetus and pregnancy outcome in female wistar rats.

Table 1: Effect of extract of *Catharanthus roseus* on body weight of pregnant rats.

Groups (mg/kg)	Initial weight (g)	Final weight (g)	Change in weight	Relative change (%)
Control	137.00±3.21	161.60±3.50	24.60±1.12	0
125	156.40±2.58	184.20±2.40	27.80±0.86	13.01
250	160.00±2.00	180.20±8.08	20.20±6.60	-17.89
500	180.00±6.51	187.20±9.33	7.20±6.47*	-70.73
1000	183.80±7.80	194.60±7.05	10.80±2.08*	-56.10

Values expressed as mean±SEM. n=5. * Significantly different from control (P<0.05).

There was an observed significant decrease in body weight of the pregnant rats. This may have been due to the numerous adverse effects of the extract on the fetus including the decrease in fetal weight. The biologically active compounds as shown in the phytochemical constituents of the extract of *Catharanthus roseus* (table 7) may have influenced the numerous biological actions of the medicinal plant.

Table 2: Effect of extract of *Catharanthus roseus* on FSH and LH levels.

Groups (mg/kg)	Progesterone (ng/ml)	Relative change (%)	Estrogen (pg/ml)	Relative change (%)
Control	49.34±2.71	0	65.60±7.13	0
125	51.04±0.79	3.45	60.60±2.18	-7.62
250	41.00±3.17	-16.90	54.00±0.84*	-17.68
500	39.94±6.45	-19.05	54.80±1.28	-16.46
1000	38.22±5.44	-22.53	60.40±3.33	-7.93

Values expressed as mean±SEM. n=5. * Significantly different from control (P<0.05).

There was a significant (P<0.05) dose dependent reductions in the levels of FSH and LH reaching a 45.95% and 56.25% decline respectively, when compared to control. Female reproductive process is regulated from the hypothalamus. Gonadotropic Releasing Hormone (GnRH) is an important hormone released by the hypothalamus to stimulate the pituitary gland to produce FSH and LH which in turn, stimulates the development of follicles (eggs) and causes the maturation of the eggs and its release in a process known as ovulation, respectively.[15]

The decrease in FSH and LH may be due to a possible inhibitory effect of extract of *Catharanthus roseus* on the hypothalamus. The extract may contain anti-gonadotropic substance which possess the ability to inhibit hypothalamic gonadotropin releasing hormone regulated anterior pituitary secretion of FSH and LH, in pregnant female rats. These changes may affect the outcome of the pregnancy. Methanolic extract of *Catharanthus roseus* contains some bioactive substances such as alkaloids. In a similar study,[16] alkaloid was reported to cause anti gonadotropic and anti progesteronic activities. The finding in the present study is consistent with those in a study,[17] that showed that *Catharanthus roseus (C.roseus)* may exhibit antifertility potential.

Table 3: Effect of extract of *Catharanthus roseus* on Progesterone and Estrogen levels.

Groups (mg/kg)	FSH (m/µ/ml)	Relative change (%)	LH (m/µ/ml)	Relative change (%)
Control	0.74±0.07	0	1.44±0.14	0
125	0.50±0.03*	-32.43	1.05±0.06*	-27.08
250	0.49±0.04*	-33.78	0.92±0.19*	-36.11
500	0.43±0.07*	-41.89	0.75±0.05*	-47.92
1000	0.40±0.04*	-45.95	0.63±0.07*	-56.25

Values expressed as mean±SEM. n=5. * Significantly different from control (P<0.05).

There was a marginal reduction in progesterone level while estrogen was significantly reduced at 250mg/kg dose of the extract administered.

During pregnancy, certain physiological and hormonal changes occur following fertilization. At the early stages, progesterone is produced by the corpus luteum, a function taken over by the placenta. Progesterone increases growth of blood vessels in the womb and allows the uterus to grow as the fetus grows etc. Both progesterone and estrogen levels increases as pregnancy progresses. Estrogens promote development of uterus, maintain the lining of uterus and also support development of the fetus.

The decrease in estrogen observed in this study agrees with the finding in another study.[18]

Hormonal irregularities may occur as a result of presence of certain compounds in plant extracts such as alkaloids and tannins. Furthermore, the extract of *C. roseus* was found to contain tannins which are a derivative of phenol. Tannins have the ability to control steroid function by inhibiting sex hormone producing enzymes which in turn can affect reproductive function at different levels.[19]

Table 4: Effect of extract of *Catharanthus roseus* on Prolactin level.

Groups (mg/kg)	Prolactin (ng/ml)	Relative change (%)
Control	0.96±0.22	0
125	0.99±0.10	3.13
250	1.18±0.08	22.92
500	1.65±0.05*	71.88
1000	1.44±0.23*	50

Values expressed as mean±SEM. n=5. * Significantly different from control (P<0.05).

Prolactin increased significantly at the higher doses of extract. Prolactin is a hormone that is secreted from the anterior pituitary gland at a usually high concentration in pregnancy. The secretion of prolactin is regulated by dopamine which is produced from the hypothalamus. Dopamine inhibits prolactin secretion.The increase in prolactin could be due to effect of *C. roseus* extract on the hypothalamic secretion of dopamine. The increase in prolactin level may have led to the significant decreases in some sex hormone levels. In a study, it was reported that highly elevated prolactin level decreases sex hormone levels. It was further concluded that high prolactin level may sensitize the hypothalamus to the negative feedback effects of gonadal steroids.

Table 5: Effect of extract of *Catharanthus roseus* on number and weights of fetus.

Groups (mg/kg)	Pregnant rats (n)	Fetus (n)	Weight of fetus (g)	% diff (in weight of fetus)
Control	5	7.40±0.68	10.68±0.72	0
125	5	9.80±0.73	10.44±0.49	-2.25
250	5	9.80±2.18	5.54±1.72*	-48.13
500	5	10.00±1.05	6.74±1.40*	-36.89
1000	5	10.80±1.77	6.70±1.55*	-37.27

Values expressed as mean±SEM. n=5. * Significantly different from control (P<0.05)

There was no significant effect of *C. roseus* on the number of fetus. However, there was a significant decrease in weight of fetuses in the groups that received higher doses (up to 250mg/kg and above).

Table 6: Effect of extract of *Catharanthus roseus* on pregnancy outcome.

Groups (mg/kg)	Pregnant rats (n)	Fetus (n)	Dead (n)	%Dead
Control	5	7.40±0.68	0.00±0.00	0
125	5	9.80±0.73	0.00±0.00	0
250	5	9.80±2.18	0.00±0.00	0
500	5	10.00±1.05	2.60±0.51*	26.00*
1000	5	10.80±1.77	3.80±0.58*	35.19*

Values expressed as mean±SEM. n=5. * Significantly different from control (P<0.05)

The number of dead fetus and the percentages of dead fetus were significantly increased in the 500 and 1000mg/kg dose of extract administered. In a study there was a reduction of pups number, body length, head length and weight of fetus considered to be due to teratogenic effect of the extract.[20] The findings in the present study is consistent with reported findings in another study.[21] which revealed that exposure of alkaloids from plants or plant products have a potential to initiate a developmental defects in humans and animals.

Table 7: Phytochemical Analysis of leaf extract of *Catharanthus roseus.*

Parameter	Indicator
Alkaloids	+++
Terpenoids	-
Flavonoids	+
Saponin	+
Anthraquinononones	-
Tannins	++
Phenols	+++
Quinine	+
Key: - = Absence of parameter; + = Presence of parameter	

CONCLUSIONS

This study has been able to show that the extract of *Catharanthus roseus* caused a dose dependent decrease in the gonadotropins; an increase in prolactin level and a consequent reduction in estrogen level. There was a significant decrease in weight of fetus and an increase in number and percentages of dead fetus with the higher doses (500 and 1000) mg/kg of the extract. These changes suggest that the extract of *Catharanthus roseus* may have abortificient potential.

REFERENCES

1. Huxley, A. New Royal Horticultural Society (RHS) Dictionary of Gardening. Macmillan ISBN, 1992; 0-333-47494-5.
2. Lewis WH,Elvin Lewis MPH, Medicinal Botany Plants Affecting Man's Health. John Wiley & Sons, New York, 1977.
3. Dessisa D preliminary economic evaluation of medicinal plants in Ethiopia: trade, volume and price In: Medhin Z, Abebe D, editors. Proceedings of the National Workshop on biodiversity Conservation and Sustainable use of Medicinal Plants in Ethiopia. Addis Ababa, Ethiopia: 28 th April-May1 st., 2001; 176-188
4. Nayak, B.S. and Pereira, L.M. *Catharanthus roseus* flower extract has wound- healing activity in Sprague Dawley rats. BMC Comp *Altern Med*., 2006; 6: 41-46.

5. Bhutkar, M.A. and Bhise, S.B. Comparative studies on antioxidant properties of Catharanthus rosea and Catharanthus alba. *Int J Pharm Tech Res*., 2011; 3(3): 1551- 1556.
6. Swati, A., Simi, J., Nikkita, C., Saloni, B., Ayesha, T., Vedamurthy, A.B., Krishna, V.& Joy, H.H. Evaluation of In-vitro Anthelminthic Activity of Catharanthus roseus Extract. *International Journal of Pharmaceutical Sciences and Drug Research*, 2011; 3(3): 211-213.
7. Singh, S.N., Vats, P. and Suri, S. Effect of an antidiabetic extract of Catharanthus roseus on enzymic activities in streptozotocin induced diabetic rats. Journal of *Ethnopharmacology*, 2001; 76: 269-77.
8. Gajalakshmi, S., Vijayalakshmi, S. & Devi Rajeswari, V. Pharmacological activities of *Catharanthus roseus*: a perspective review. *International Journal of Pharma and Bio Sciences*, 2013; 4(2): 431–439.
9. Cooper, R. and Deakin, J.J. Botanical miracles: chemistry of plants that changed the world. CRC Press., 2016.
10. Mustafa, N.R.and Verpoorte, R. Phenolic compounds in *Catharanthus roseus* . *Phytochemistry Reviews*, 2007; 6: (2-3): 243–258. doi: 10.1007/s11101-006-9039-8.
11. Abasiubong, F., Bassey, E.A., Udobang, J.A., Akinbami, O.S., Udoh, S.B and Idung, A.U Self-Medication: potential risks and hazards among pregnant women in Uyo, Nigeria. The Pan African Journal, 2012. PMCID: PMC3527026; PMID: 23308320
12. Titilayo, O.F; Rasaq, A. and Ismail, E.M (2009). Attitude and use of herbal medicines among pregnant women in Nigeria. BMC *complementary and alternative medicine*, 2009; 9(53).
13. National Institute of Health (N.I.H). Guide for the care and use of laboratory animals. DHEW Publication. Office of Science and Health Reports, 1985; Bethesda, U.S.A.
14. Kabubii, Z.N., James, M.M. and Mathiu, M. Acute toxicity studies of *Catharanthus roseus* aqueous extract in male Wistar rats. *African Journal of Pharmacology and Therapeutics*, 2015; 4: 130-134.
15. Hafez, B. and Hafez, E.S.E. Reproduction in farm animals, 7th Edition, Lippincott Williams and Wilkins, 2000; 3-95.
16. Musa**,** T.Y and Isa**,** F.M**.** Effects of Post-coital Administration of Alkaloids from Senna alata (Linn. Roxb) Leaves on some Fetal and Maternal Outcomes of Pregnant Rats. Journal of reproduction and infertility, 2012; 13(4): 211-7. PMID: 23926548PMCID: PMC3719345
17. Mathur, R. and Chaudan, S. Antifertility efficacy of *Catharanthus roseus* Linn: a biochemical and histological study. ActaEur Fertility, 1985; 16(3): 203-205.
18. Preeti, G. Antiestrogenic Activity of Petroleum Ether Extract of the Leaves of Catharanthus roseus (Vincarosea) in Female Albino Mice *Asian J. Exp. Sci.*, 2009; 23(1): 313-316.
19. Nesrein, M.H., Antonio, G.B. and Jesus, S.G. Polyphenols in Farm Animals: Source of Reproductive Gain or Waste?. Anti oxidants MDPI, 2020.
20. Bushra, M.A. M. and Sherzad, I.M.A. An investigation of the Cytotoxic, Cytogenetic and Teratogenic Potentials of *Catharanthus roseus*. *International Journal of Science and Research* (IJSR) ISSN (Online), 2015; 2319-7064: 6.391.
21. Benedict, T.G., Stephen, T.L., Kevin, D.W. & Kip, E.P. Plant alkaloids that cause developmental defects through the disruption of cholinergic neurotransmission. Birth Defects Res C Embryo Today. Dec., 2013; 99(4): 235-46.doi: 10.1002/bdrc.21049.