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The Influence of Methanol Extracts of Some Plant Species Used in the Management of Pregnancy-Related Symptoms on the Reproductive Parameters of Pregnant Wistar Rats

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ABSTRACT

Herbal medicines are increasingly being used during pregnancy and little is known of their potential teratogenic effect. The present study is therefore designed to investigate the teratogenic effects of methanol extracts ofthree medicinal plants; *Jatropha curcas*, *Alchornea cordifolia*, and *Secamone afzelii* in Wistar rats. The animals were administered the extractsat doses of 50, 100, and 200 mg/kg.Morphometry of the placenta and foetus were recorded, the weight, crown-rump length, head circumference and anogenital distance were measured. Acute toxicity of the extracts was also evaluated. Embryo resorptions in some treated rats as well as foetal malformation were observed. In the acute toxicity test, at the maximum dose of 5000 mg/kg, the methanol extracts of the three plants; *Jatropha curcas*, *Alchornea cordifolia*, and *Secamone afzelii* showed no harmful effects on the pregnant rats. However, strong teratogenic effects were noted. At doses higher than 50mg/kg, *Alchornea cordifolia* extract-exposed pregnant rats produced 7 dead foetuses out of 9 implantations; the live foetuses were deformed, particularly in their limbs. Animals administered with *Secamone afzelii* had 11% foetal mortality at 100 and 200mg/kg dose. No dead foetus or foetal deformity wasobserved in the rats administered with 50, 100, and 200 mg/kg *Jatropha curcas*extract. Although the methanolextracts of the plants used in this study were not acutely toxic to pregnant Wistar rats at the doses tested, they caused some teratogenic effects, especially with *Alchornea cordifolia* extracts.

Keywords: Teratogenicity, Acute toxicity, Plant extracts, Animal model, Phytomedicinals.

Introduction

Herbal medicines are gaining more popularity in both developing and developed countries, despite the fact that their use and safety, particularly during pregnancy, are poorly understood.[1] Herbal or traditional medicine, as it is commonly known, is used by an estimated 80% of the population in rural parts of developing nations for their health care needs, particularly during pregnancy.[2] While herbal therapies varies from country to country, many of the same herbs are used.[3] Evidence from Africa shows a wide range of herbal medication use during pregnancy, for example, one Nigerian study showed 68% and another study indicated 12% of herbal medicine use during pregnancy.[4,5]A study shows that 21% percent of pregnant women in Zambia's public health system use traditional remedies.[6] As a result, utilizing herbal remedies instead of scientifically approved treatments during pregnancy may have serious consequences, such as foetal distress and premature births,[7] intrauterine growth restriction, and low foetal survival, and congenital abnormalities,[8] among others.

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Herbal drugs can have the potential to affect the uterus, perhaps resulting in abortion.[9]Herbal products are used often during pregnancy despite the fact that little or nothing is known about the potential negative effects of many herbal drugs during pregnancy.

The growing dissatisfaction among patients about the safety, effectiveness, and efficacy of standard allopathic drugs has led to an increase usage of herbal medicines for the treatment of both mild and major illnesses.[2] Herbal drugs have been used to treat a wide range of pregnancy-related issues, including nausea, vomiting, acid reflux, and candida vaginal infections, to name a few. Herbal drugs may be used to treat chronic conditions, such as hypertension. According to Osamor and Owumi,[10] 29% of the population in urban Nigeria has at one point or the other taken plant extracts for hypertension. Kaingu *et al*.[11] described Traditional Birth Attendants' use of herbal products to treat both prenatal and postpartum issues.

Regardless of how prevalent the use of herbal treatments in pregnancy care is, the issue of toxicity always arises. Most of the time, cumulative effects might result in toxicity, which can include carcinogenic, mutagenic, or teratogenic effects.[12-14]Despite the deleterious effects they might have on humans and animals when used, various studies have shed light on the bioactivities of traditionally used therapeutic herbs.[12,15] Secondary metabolites from these herbs may cause lethal or non-lethal toxicity.[13,14,16]

According to Mendes *et al*.[17] aqueous and ethanol extracts of *Bauhinia monandra* had no discernible negative effects on pregnant rats. There are no established criteria for the use of plant-based medicinal treatments in most countries, and practitioners rarely have access to science-based information. Herbal side effects are more likely to affect people who are already predisposed to experiencing negative effects from traditional pharmaceutical formulations. Those who are more susceptible to these effect are pregnant women, infants, teenagers, nursing mothers as well as the elderly.[18,19] This study examined the

acute toxicity and teratogenic potential of *Secamone afzelii* , *Alchornea cordifolia*, and*Jatropha curcas* methanol extracts in pregnant Wistar rats. These plants are often used locally to treat pregnancy-related symptoms such as high blood pressure, nausea, and vomiting.

Materials and Methods

Reagents
All reagents used were of analytical grade (Lob PVT, Mumbai, India; Guangdong, GuamghuaSci-Tech Co Ltd China).

Plant Sample Collection and Processing
The leaves of *Secamone afzelii* , *Alchornea cordifolia*, and *Jatropha curcas* were collected in January, 2019 from a commercial vegetable farm in Iguosula, Edo State. The plant materials were identified and authenticated in the Phytomedicine Unit of the Department of Plant Biology and Biotechnology, University of Benin. Herbarium specimens were deposited in the unit with voucher numbers UBH-J404, UBH-A560, and UBHS566 for *Jatropha curcas*, *Alchornea cordifolia*, and *Secamone afzelii* , respectively.The leaves were washed with distilled water and allowed to air-dry for two weeks. The dried leaves were powdered with the aid of a kitchen blender (Panasonic® MX-GX1021WTZ).The powdered leaves (100 g each) were extracted by macerationin 200 mL of methanol for 12 h. The extract was filtered with a Whatman filter paper #42 (125 mm), and then evaporated to dryness.

Animals
Female Wistar rats weighing between 220 and 256 g were used for the study. The animals were housed in well ventilated cages at room temperature with ambient light and dark cycle. The rats were fed with regular rodent feed (0.35 g NaCl, 20 g protein, and 1.17 g arginine per 100 g feed) and had access to drinking water *ad libitum*. The animals were acclimatized to the laboratory conditions for one week prior to the experiment. The animals were divided into two groups; the first group for acute toxicity study and rats in the other group were mated with male rats until pregnancy was established, and then used for the teratogenic studies.

Ethical Issues
Ethical approval for the study was granted by the Faculty of Life Sciences Research and Ethics Committee with reference number LS19017.

Acute Toxicity Screening
Except for the control group, the animals were administered oral doses of the extractsat 10, 100, 500, 1000, and 5000 mg/kg body weight once daily for 15 days. Mortality and morbidity were recorded on days 1, 3, 6, 9, 12, and 15.[20]After day 15 of the treatment, the animals were sacrificed under chloroform anaesthesia.

Measurement of Teratogenic Parameters
After the animals have been sacrificed, morphometryof the placenta, uterus, as well as the presentations of the foetuses were recorded. The placentalweight was measured using a digital precision analytical balance. Volume of the placenta was determined using Archimedes Principle whereby the volume of water in a measuring cylinder displaced after adding the placenta was taken as the volume of the placenta. Placenta circumference was measured by encircling the placenta with a threadand then measuring the thread with a millimeter rule. The weight, circumference and volume of the placenta were used to assess the rate of growth of the foetus in-utero.[21]
Morphometric parameters of the foetus measured included weight, crown-rump length, head circumference and anogenital distance. Crown-rump length (CRL) was measured as the distance from the tip of the head to the anus of the rats, whereas anogenitaldistance (AGD) was taken as the distance from the end of the penis to the anus.[22] Both CRL and AGD were measured using a vernier caliper. Crown-rump lengthwas used to assess physical growth, while AGD was used to assess the development of the reproductive system. Head circumference was used to assess the development of the brain.
The terms "foetal resorption" and "embryo resorption" are interchangeable terms describing the disintegration of an embryo that is already forming in the uterus. Although embryo resorption occurs before organogenesis (formation of organ) while foetal resorption occurs after organogenesis.[23]

Statistical analysis
SPSS Version 20 and GraphPad Prism Version 5 were used for data analysis. Data were presented as mean ± standard error of mean (SEM). Differences between means were determined using least significant differences at a 95% probability level.

Results and Discussion

The World Health Organization (WHO) in 2002 suggested that medicinal plants is the primary source of a variety of medicines.[2] As a result, such medicinal plants should be examined thoroughly in order to gain better understanding of their therapeutic qualities, safety, and efficacy.[24]
Many people, however, consume medicinal plants based on personal experience or traditional knowledge, and it is unclear if these plants are safe for human consumption during pregnancy. Previous research has shown that several plants have antifertility properties even though they initially did not cause acute toxicity.[25] The present study investigated the potential teratogenic effects of the methanol leaves extracts of *Jatropha curcas*, *Secamone afzelii* , and *Alcornea cordifolia* even though they may have been declared safe by virtue of their negative acute toxicity effect in Wistar rats.
Figure 1 shows the acute toxicity effects of the test plants. The results showed that at 5000 mg/kg dose, all three plant extracts did not causeany toxicity or mortality in the Wistar rats.
Table 1 shows the external morphology of the foetus of the test animals after exposure to the extracts. The results showed that the foetal circumference was 4.02 mm in the control animals; whereas, the foetal circumference was significantly reduced to 3.22 mm on treatment with 50 mg/kg *Jatropha curcas* extract. There were no significant reductions in CRL and AGD as well as total weight. Administration of extracts of *Alchornea cordifolia* resulted in minimal reduction in foetal circumference ($p>0.05$). However, significant increase in CRL was observed.
The external morphology of the placenta of the test animals are presented in Table 2. There were minimal changes in placental circumference ($p>0.05$). Placental circumferences ranged from 3.8 – 5.1 mm ($p>0.05$) irrespective of the extracts administered. Similarly, no significant changes in weight of placenta (0.4 - 0.5g) as well as the weight of uterus (3.7 – 4.9g) was observed in the test group compared to the control. However, the administration of 50 mg/kg extracts of *Secamone afzelii* resulted in a significant increase in placental volume (0.53 – 0.56 mL) compared to the control (0.39 mL). Administration of extracts of *Jatropha curcas* and *Alchornea cordifolia* did not show any significant effect on placental volume.
The effects of the administration of the extracts on foetal formation showed that with 50 - 200 mg/kg *Jatropha curcas*, no foetal mortality was recorded, neither were there any deformity or resorption (Table 3). However, administration of 100 mg/kg *Secamone afzelii* extract resulted in 11.11% foetal mortality. At doses above 50mg/kg of *Alchornea cordifolia* extract, pregnant Wistar rats produced 7 dead foetuses out of 9 implantations; the live foetuses were deformed, particularly in their limbs.
Foetal development is a precise process that combines molecular and cellular changes with precise timing to produce various phenotypes throughout the organism. During pregnancy, foetal development is assessed using a variety of maternal indicators, including fluctuations in body weight, the level of food intake, quantity of water consumed, and other nutrition consumptions necessary for subsistence. Other forms of clinical toxicity indicators like hypersalivation, uncontrolled bowel movements, behavioural disorders, and genital bleeding are measures of maternal homeostasis.[26-28]An increase in

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maternal body weight is typically associated with pregnancy, the fact that the dams in this study showed such an increase indicated that they were pregnant.

The procedure of insertion of the fertilized egg is critical in the development of the foetus; the reproductive capacity index is connected with the site of implantation and the extent of the corpus luteum. The quantity of established and underdeveloped blastocysts is the focus of the resorption index and the post-implantation loss rate, in contrast to the implantation index and the pre-implantation loss rate, which measure how deeply a blastocyst is implanted in the uterus.[26,29] In the current study, there were 8 implantations in the control group, but in the extracts treated groups, there were minor variations in the number of implantations compared to the control group. However, regardless of concentration, administration of *Alchornea cordifolia* extracts resulted in considerable foetal death and malformation in those who survived. Previous studies have also shown that daily administration of *Alchornea cordifolia* root aqueous extract during the first trimester of pregnancy in female rats had an impact on the transplantation rate in comparison to the control group.[30,31]Similarly, the study by Goonasekera *et al*.[32]found a decrease in embryo transfer sites following the administration of *Jatropha curcas* fruit extract. Phytochemicals, hormones and uterine activity have been demonstrated to disrupt pregnancy by interfering with embryo development during the mitotic phase, resulting in embryonic loss.[33,34] Inactivation and improper positioning of blastocytes associated to uterine activity are anticipated to be detrimental to embryo implantation.[35]

The results from the present study suggest that *J. curcas*extract at doses of 50 – 200 mg/kg, and *S. afzelii*extract at 50 mg/kg dose may be safe for use in pregnancy (Table 4).

Abortion-inducing alkaloids have been used for ages, and the presence of this phytochemical may be responsible for the arbortificient properties of *Alchornea cordifolia*. Researchers have hypothesized that hormonal imbalance could result in fewer implant sites. Progesterone and estrogen levels must be precisely balanced for implantation, and it is widely acknowledged that alterations to this ratio result in infertility.[36-38] It has also been reported that progesterone levels in the blood rise during pregnancy to maintain the conditions for the foetal growth and development. Thus, lower serum progesterone in pregnancy may have unintended repercussions such as embryonic resorption, abortion, and implantation inhibition.[31,39]

In female rats, the sequence of events leading to implantation (the initial stage of pregnancy) is tightly regulated.[40]As a result,a slight alteration in this regulatory mechanism might cause endometrial dysfunction. Early in pregnancy, high dose administration of an aqueous extract of *Alchornea cordifolia* lower blood progesterone levels, causes embryo resorptionand a total failure of implantation.

Flavonoids, alkaloids, saponins, steroids, and terpenoids have all been found to have contraceptive properties.[40-43]Therefore, the abortive and pregnancy-terminating actions of *Alchornea cordifolia* extract in late-gestational female rats may be due to the presence of one or more of these phytochemicals. These chemicals may work together in treated female rats to cause foetal death, abortion, and vaginal bleeding. Despite the fact that none of the extracts showed substantial acute toxicity, the teratogenicity of *Alchornea cordifolia* extracts made it unsafe to use.

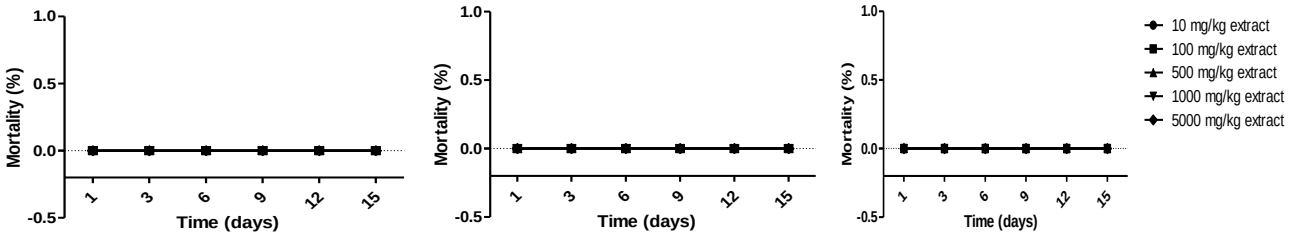

Figure 1: Percentage mortality of rats used during acute toxicity study (a)*Jatropha curcas*(b)*Alchornea cordifolia,* and (c)*Secamone afzelii*

Table 1: External Morphology of foetus of test animal after exposure to extracts

Extract (n = 10)	**Foetal Circumference (mm)**	**CRL**	**AGD**	**Weight (g)**
Control	4.02	2.87	0.28	0.89
50 mg/kg *Jatropha curcas*	3.22*	2.79	0.24	0.99
100 mg/kg *Jatropha curcas*	3.12*	3.04	0.31	0.89
200 mg/kg *Jatropha curcas*	3.24*	3.14	0.28	0.93
50 mg/kg *Alchornea cordifolia*	3.41	4.51*	0.37	2.08*
100 mg/kg *Alchornea cordifolia*	3.52	4.98*	0.45	2.11*
200 mg/kg *Alchornea cordifolia*	2.74*	4.03	0.39	1.59
50 mg/kg *Secamone afzelii*	3.99	5.01*	0.28	0.94
100 mg/kg *Secamone afzelii*	4.02	5.11*	0.28	1.03
200 mg/kg *Secamone afzelii*	3.87	4.78*	0.31	1.14
LSD(0.05)	0.64	1.33	0.18	0.86
p-value	0.001	<0.001	0.073	0.021

* Means are significantly different from the control ($p<0.05$)
CRL = Crown–rump length; *AGD*= Anogenital distance

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Table 2: External morphology of Placenta after exposure of test animals to extracts

Extract (n = 10)	Circumference (mm)	Weight (g)	Volume (mL)	Wt. of uterus (g)
Control	4.1	0.5	0.39	3.9
50 mg/kg *Jatropha curcas*	4.4	0.5	0.41	3.8
100 mg/kg *Jatropha curcas*	4.7	0.4	0.43	3.6
200 mg/kg *Jatropha curcas*	3.9	0.5	0.39	3.3
50 mg/kg *Alchornea cordifolia*	3.5	0.4	0.31	3.9
100 mg/kg *Alchornea cordifolia*	4.1	0.4	0.38	4.1
200 mg/kg *Alchornea cordifolia*	3.8	0.4	0.41	3.7
50 mg/kg *Secamone afzelii*	4.6	0.5	0.53*	4.7
100 mg/kg *Secamone afzelii*	5.1	0.5	0.56*	4.9
200 mg/kg *Secamone afzelii*	4.2	0.4	0.53*	4.3
LSD(0.05)	0.7	0.2	0.13	1.1
p-value	0.085	<0.001	<0.001	0.441

* Means are significantly different from the control (p<0.05)

Table 3: Effect of extracts on foetal formation

Extract	Number of Implantations	Number of Live foetuses	Number of Dead foetuses	Average percentage mortality (%)	Deformed	Resorptions
Control	8	8	0	0	0	0
50 mg/kg *Jatropha curcas*	9	9	0	0	0	0
100 mg/kg *Jatropha curcas*	7	7	0	0	0	0
200 mg/kg *Jatropha curcas*	9	8	0	0	0	0
50 mg/kg *Alchornea cordifolia*	9	2*	7	77.78	9	0
100 mg/kg *Alchornea cordifolia*	9	2*	7	77.78	9	0
200 mg/kg *Alchornea cordifolia*	9	3*	5	55.56	8	0
50 mg/kg *Secamone afzelii*	9	8	0	0	0	0
100 mg/kg *Secamone afzelii*	9	8	1	11.11	0	0
200 mg/kg *Secamone afzelii*	9	7	1	11.11	0	0
LSD(0.05)	3	3	NA	-	NA	NA
p-value	0.296	<0.001	NA	-	NA	NA

Means are presented to the nearest integer
*Means are significantly different from the control (p<0.05)

Table 4: Decision on herbal safety based on teratogenicity

Extract concentration	Toxicity		Remark
	Teratogenicity	Acute	
50 mg/kg *Jatropha curcas*	-	-	Safe for use
100 mg/kg *Jatropha curcas*	-	-	Safe for use
200 mg/kg *Jatropha curcas*	-	-	Safe for use
50 mg/kg *Alchornea cordifolia*	+	-	Unsafe
100 mg/kg *Alchornea cordifolia*	+	-	Unsafe
200 mg/kg *Alchornea cordifolia*	+	-	Unsafe
50 mg/kg *Secamone afzelii*	-	-	Safe for use
100 mg/kg *Secamone afzelii*	+	-	Unsafe
200 mg/kg *Secamone afzelii*	+	-	Unsafe

Conclusion

The study has shown that the methanol extracts of *Jatropha curcas*, *Alchornea cordifolia*, and *Secamone afzelii* were not acutely harmful to pregnant Wistar rats, but they caused some teratogenic effects, particularly with extracts of *Alchornea cordifolia*. The deformities seen were morphometric variations in the placental weight and volume, Crown-rump length and Anogenital distance. These values for these parameters were lower in most animals in the treatment group compared to the control group.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Ernst E. Herbal medicinal products during pregnancy: are they safe? Br J Obs Gyn. 2002; 109:227–235.
2. World Health Organization (WHO). Traditional Medicine Fact Sheet No.134, 2008. http://www.who.int/mediacentre/factsheets/2003/fs134/en/. Accessed 29 June, 2016.
3. Kennedy DA, Lupattelli A, Koren G, Nordeng H. Herbal medicine use in pregnancy: results of a multinational study. BMC Complement Altern Med. 2013; 13:355.
4. Gharoro EP and Igbafe AA. Pattern of drug use amongst antenatal patients in Benin City, Nigeria. Med SciMonit. 2000; 6:84–87.
5. Yolan B, Victoria C, Robert LG, Jeffrey SAS, Jennier FC, Moses S. Use of traditional medicine among pregnant women in Lusaka, Zambia. J Altern Complement Med. 2007; 13(1):123–128.
6. Mudonhi N and Nunu WN. Traditional Medicine Utilisation Among Pregnant Women in Sub-saharan African Countries: A Systematic Review of Literature. Inquiry.2022; 59:469580221088618.
7. Chan LY, Chiu PY, Lau TK. An *in-vitro* study of guisenoside Rb1-induced teratogenicity using a whole rat embryo culture model. Hum Reprod. 2003; 18(10):2166–2168.
8. Gruber CW and O'Brien M. Uterotonic plants and their bioactive constituents. Planta Med. 2011; 77(3):207–220.
9. Osamor PE and Owumi BE. Complementary and alternative medicine in the management of hypertension in an urban Nigerian community. BMC Complement Altern Med. 2010; 10-36.
10. Kaingu CK, Oduma JA, Kanui TI. Practices of traditional birth attendants in Machakos District, Kenya. J Ethnopharmacol. 2011; 137(1):495–502.
11. Nath P and Yadav AK. Acute and sub-acute oral toxicity assessment of the methanolic extract from leaves of Hibiscus rosa-sinensis L. in mice. J Intercult Ethnopharmacol. 2015; 4:70–73.
12. Kilonzo M, Ndakidemi PA, Chacha M. *In vitro* antifungal and cytotoxicity activities of selected Tanzanian medicinal plants. Trop J Pharm Res. 2016; 15:2121–2130.
13. Jatsa HB, Fassi J, Kenfack MMC, Feussom NG, Kameni M, Simo NND, Nkondo ET, Dongmo AB, Tchuente LT. Acute and sub chronic oral toxicity studies of the leaves aqueous extract of *Clerodendrum umbellatum* Poir. On mice. Am J Physiol Biochem Pharmacol. 2018; 7:75–85.
14. Bello I, Bakkouri AS, Tabana YM, Al-hindi B, Al-Mansoub MA, Mahmud R, AsmawiMZ. Acute and sub-acute toxicity evaluation of the methanolic extract of *Alstonia scholaris* stem bark. Med Sci. 2016; 4:1–14.
15. Toghueo RMK. Bioprospecting endophytic fungi from Fusarium genus assources of bioactive metabolites metabolites. Mycol. 2019; 91:1–21.
16. Mendes CC, Marinho CM, Moreira-Junior VF, Danta GL, Macedo MF, Oliveira CN, Schwarz A. Evaluation of *Bauhinia monandra* aqueous and ethanol extracts in pregnant rats. Pharm Biol. 2010;48(7):780-785.
17. Roulet M, Laurini R, Rivier L, Calame A, Hepatic veno-occlusive disease in newborn infant of a woman drinking herbal tea.J Pediatr1988; 112:433-436.
18. Holcberg G, Tsadkin-Tamir M, Sapir O, Mahmoud H, Moshe M, Ben Z. New Aspects in Placental Drug Transfer. Israel Med Assoc J. 2003; 5:873-876.
19. Adeyemo-Salami OA and Makinde JM. Acute and sub-acute toxicity studies of the methanol extract of the leaves of *Paullinia pinnata* (Linn.) in Wistar albino mice and rats. Afr J Med Med Sci. 2013; 42(1):81-90.
20. Alcala M, Bolado, VE, Sánchez-Vera I, Clapés S, Dasí F, Sáez G, Carrera E, Alvarez-Gallego F, Loeken MR, Viana M. Prevention of Teratogenesis in Pregnancies of Obese Rats by Vitamin E Supplementation. Antioxid. 2021; 10:1173.
21. Najdi N, Safi F, Hashemi-Dizaji S, Sahraian G, Jand Y. First trimester determination of fetal gender by ultrasonographic measurement of anogenital distance: A cross-sectional study. Int J Reprod Biomed. 2019; 17(1):51-56.
22. Flores LE, Hildebrandt TB, Kühl AA, Drews B. Early detection and staging of spontaneous embryo resorption by ultrasound biomicroscopy in murine pregnancy. ReprodBiolEndocrinol. 2014; 12:38.
23. Nascimento-Antman EM and Braunwald E. ST-elevation myocardial infarction: pathology, pathophysiology, and clinical features. In: Libby P, Bonow RO, Mann DL,Tomaselli GF, and Braunward E. eds. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. Philadelphia, PA: Saunders Elsevier. 2008. 1207-1232 p.
24. Priya G, Saravanan K, Renuka C. Medicinal plants with potential antifertility activity- A review of sixteen years of herbal medicine research (1994-2010). Int J Pharm Tech Res. 2012; 4:481-494.
25. Almeida FCG and Lemonica IP. The toxic effects of *Coleus barbatus* B. on the different periods of pregnancy in rats. J Ethnopharmacol. 2007; 73:53–60.
26. Butte NF, Wong WW, Treuth MS, Ellis KJ, Smith EOA. Energy requirements during pregnancy based on total energy expenditure and energy deposition. Am J Clin Nutr. 2004; 76(6):1078–1087.
27. Guerra M, Mazoni ASB, Brandao MAF, Peters VM. Toxicology of lapachol in rats: Embryo lethality. Rev Braz Biol. 2000; 61:71-174.
28. Ekhator CN and Shelu JO. An Experimental Study on the Abortificient Potentials of Unripe Seed Extract of *Carica papaya* in Adult Female Wister Rats, Open Sci J Pharm Pharmacol.2015; 3(6):61-65.
29. Loebstein R, Lalkin A, Oren GK. Pharmacokinetic changes during pregnancy and their clinical relevance. ClinPharmacokin.1997; 33(5):328-343.
30. Yakubu MT and Bukoye BB. Abortifacient Potential of the Aqueous Extract of *Bambusa vulgaris* Leaves in Pregnant Dutch Rabbits. Contraception. 2009; 80:308-313.
31. Yakubu MT, Olawepo OJ, Fasoranti GA. *Ananascomosus*: Is the Unripe Fruit Juice an Abortifacient in Pregnant Wistar Rats? Eur J Contracept Reprod Health Care. 2011; 16:397-402.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

32. Goonasekera MM, Gunawardana VK, Jayasena K, Mohammed, SG, Balasubramaniam, S. Pregnancy Terminating Effect of *Jatropha curcas*in Rats. J Ethnopharmacol. 1995; 47:117-123.
33. Elbetieha A, Oran SA, Alkofahi A, Darmani H, Raies AM. Fetotoxic Potentials of *Globularia arabica* and *Globularia alypum* (Globulariaceae) in Rats. JEthnopharmacol. 2000; 72:215-219.
34. Chen Q, Zhang Y, Lu J, Wang Q, Wang S, Cao Y. Embryo-Uterine Cross-Talk during Implantation: The Role of Wnt Signaling. Molecul Hum Reprod. 2009; 15:215-221.
35. Elderfied RC. Biographical Memoir for Walter Abraham Jacobs, 51. National Academy of Science, Washington DC.1980. 247-278 p.
36. Spencer TE, Johnson GA, Burghardt RC, Bazer FW. Progesterone and Placental Hormone Actions on the Uterus: Insights from Domestic Animals. BiolReprod.2004; 71:2-10.
37. Pooja S, Prashanth S, Suchetha K, Vidya V, Krishna B. Evaluation of acute and sub acute toxicity of the leaf extract of *Tanacetum parthenium* (Asteraceae) and synthetic parthenolide. World J Pharm Pharm Sci. 2016; 5(8):703-713.
38. Azadeh M, Maryam P, Mozafar K,Rostam G. Anti-Fertility Effects of *Physalis alkekengi* Alcoholic Extract in Female Rat. Iran J Reprod Med. 2007; 5:13-16.
39. Titilayo OK, Rasaq A, Ismail EM. Attitude and use of herbal medicines among pregnant women in Nigeria. BMC Complement Altern Med. 2009; 9:53.
40. Hiremath SP, Rudresh K, Badami S, Patil SB, PatilRS. Post-Coital Antifertility Activity of *Acalypha indica* L. J Ethnopharmacol. 1999; 67:253-258.
41. Ravichandra V, Suresh B, Satishkumar MN, Elango K, Srinivasan R. Antifertility Activity of Hydroalcoholic Extract of *Ailanthus excels* (Roxb): An Ethno Medicine Used by Tribes of Nilgiris Region in Tamilnadu. J Ethnopharmacol. 2007; 112:189-191.
42. Zanwar AA, Aswar UM, Hegde MV, Bodhankar SL. Estrogenic and Embryo-Fetotoxic Effects of Ethanol Extract of *Linum usitatissimum*in Rats. J Complement Integr Med.2010; 7:1-17.
Uchendu CN, Kamalu TN, Asuzu IU. A Preliminary Evaluation of Antifertility Activity of a Triterpenoid Glycoside (DSS) from *Dalbergia saxatilis* in Female Wistar Rats. Pharmacol Res. 2000; 41:521-525.