ORIGINAL COMMUNICATION

EMBRYOFETAL EFFECTS OF THE METHANOLIC ROOT EXTRACT OF *CISSAMPELOS MUCRONATA* A. RICH IN RATS

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ABSTRACT

This study was designed to examine the effect of the methanolic extract of the root of *Cissampelos mucronata* on embryo-foetal development in rats. A total of 60 nulliparous female rats weighing 143-215gm approximately 13 weeks of age in the proestrous stage were cohabited (1:1) with 60 male rats to obtain 40 pregnant rats which were assigned to four dosage groups (10 rats per group, I-IV) by random stratification to nearly equalized body weight difference between groups on gestataion day zero GD 0. Group I served as the control group while rats in Groups II, III and IV were administered with 100mgkg^{-1}, 200mgkg^{-1}and 300mgkg^{-1} doses of the extract respectively from implantation (GD6) to the day prior to the expected day of parturition (GD20). On GD20, the female rats were euthanized and uterine implantation data including live and dead foetuses were counted, weighed, gender determined and observed for external malformations. The results indicate that administration of the extract from gestation day 6 to 20 had no significant effect on the number of implantation sites while resorptions sites were significantly ($p<0.05$-0.001) high in a dose dependent manner. There was a decrease in the foetal weight, placental weight and crown rump length of the rats indicating that administration of the extract from gestation day 6 to 20 caused resorptions in pregnancy and decrease in foetal weight, placental weight and crown rump length rats in a dose dependent manner.

Key words: Embryotoxic, crown rump, resorption, viable, foetus

INTRODUCTION

Cissampelos mucronata (A. Rich) belongs to the family Menispermaceae and is highly specialized in its extraordinarily rich diversification of benzyltetrahydroisoquinoline and aporphine derivatives (Watson and Dallwitz, 1992). Because of its richness in alkaloids, this family is used worldwide in traditional medicine to treat a variety of ailments. It is used to facilitate childbirth, as an abortifacient, as an emmenagogue, to treat stomach pains and as a diuretic (Burkill , 1997). Its fresh aerial parts are used to regulate menstruation (Elujoba, 1995). Ash obtained from the leaves, twigs and root bark are eaten to treat side pain (Baerts and Lehmann, 1989) and the sap of the whole plant is mixed with *Heliotropium indicum* L. and drunk to treat Leukorrhea (Adjanohoun *et al.*1989). Decoction of the stem bark and leaves is also drunk to calm nerves (Nwosu, 1999). The root is used to treat abdominal pains, swollen stomach and gastro-intestinal upset due to bewitchment (Gelfand *et al.* 1985; Chhabra *et al.* 1990). It is also used to prevent or arrest uterine haemorrhage, painful uterus, to treat infertility, to prevent abortion (Gelfand *et al.*1985; Van Wyk and Gericke, 2000) and to treat conjunctivitis (Tshibangu *et al.* 2002) .

In Nigeria, like many African countries *Cissampelos mucronata* (A. Rich) is usually prepared in the form of infusions, decoctions, tinctures or syrups in the treatment of various ailments. Its indigenous names in Nigeria include *Jibdar Kasa* or *Damarji* (Hausa) and abakenwo in Igbo (Burkill, 1997), *Barwada* (Kanuri), *Magirahi* (Fulfulde), *Zagaduwa* (Marghi) and *Kwahara* or *Kwahirka* (Babur/Bura) while its English name is Ivy vine. Despite its various uses during pregnancy a search conducted for embryofetal (developmental) effect of *Cissampelos mucronata* found no results. Against this background the study was designed to examine the effect of the methanolic extract of the root of *Cissampelos mucronata* on embryo-foetal development in rats according to standard methods.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The plant was collected around Giwa Military Barracks in Maiduguri metropolis latitude 11° 50' 42" North and longitude 13° 9' 36" East. The plant was identified and authenticated as *Cissampelos mucronata* A. Rich by Professor S. S. Sanusi (botanist) of the Department of Biological Sciences, University of Maiduguri, Borno state, Nigeria. A specimen voucher (CM.01) of the plant was prepared and deposited at the herbarium of the Department of Veterinary Physiology and Pharmacology, University of Maiduguri, Borno state. The collection, identification and storage of the plant material was carried out according to the World Health Organization's guidelines on quality control methods for medicinal plant materials (WHO, 1998) and quality specifications of plant materials and preparations (WHO, 1993). The root was then sun-dried, pulverised into powdered form using a pestle and mortar and then stored in cellophane bags at room temperature.

Extraction Procedures

The World Health Organization's research guidelines for evaluating the safety and efficacy of herbal medicines (WHO, 1993) was adopted for the extraction of the root of *Cissampelos mucronata*. A total of one hundred grams (100g) of the pulverised root was subjected to exhaustive soxhlet extraction in methanol (500ml) for 72 h at 60^{o}C. The extract obtained was then concentrated in a water bath until a constant dark sticky residue was obtained, this gave a total mean extract weight yield of 11.34g w/w of extract which was further oven dried and maintained in a desiccator until a constant weight was obtained and the extract obtained was then stored in a stoppered container in refrigerator at - 4^{o}C until required.

The stock solution of the extract was prepared by dissolving a known weight (2g) of the extract in a known volume of distilled water (50ml) in the presence of 1 drop (0.05ml) of dimethylsulfoxide (DMSO) and allowed to stand for about 45-60 minutes, the water soluble portion was separated off using a fine needle syringe and the dry weight of the marc determined after drying the wet marc on a hot plate. The actual concentration of the water soluble portion of the extract was thus determined and required concentrations were then prepared from the stock solution by serial dilutions.

Embryofetal study

A total of 60 nulliparous female rats weighing 143-215gm and approximately 13 weeks of age and 60 male rats weighing 165-220 gm that have not being subjected to any experimental

procedure were used in this study and cared for according to the Guiding Principles for the Care and Use of Animals based on the Helsinki Declaration as amended by World Medical Assembly, Venice, Italy (Declaration of Helsinki, 1996). The study was conducted according to methods outlined by U.S, EPA, 1998; U.S.FDA, 2000 and ICH, 2005. Female rats that were in the proestrous stage of the oestrous cycle were cohabited (1:1) with the males and were considered pregnant based on the presence of sperm cells or a vaginal plug. The experiment was started with 60 female rats in order to achieve the minimum and sufficient number of 10 pregnant rats per group (experimental and control) thus, the 40 pregnant rats obtained were assigned to four dosage groups (10 rats per group, I-IV) by random stratification to nearly equalized body weight difference between groups on GD 0.

Group I served as the control group and were administered normal saline equivalent to the volume administered to the highest dosed experimental rats while rats in Groups II, III and IV were administered with 100mgkg^{-1}, 200mgkg^{-1}and 300mgkg^{-1} doses of the extract respectively (Dosages were calculted based on an initial acute toxicity study that gave an LD_{50} of above 2000mg/kg/oral.). The rats were administered the extract daily by gavage from implantation (GD6) to the day prior to the expected day of parturition (GD20) and observed daily for mortality and toxicological effects. On GD20, the female rats were euthanized, a caesarean section performed and macroscopic post-mortem examination performed on the uterus and ovaries.

Uterine implantation data including live and dead foetuses were obtained from the uterus, counted, weighed, gender determined and observed for external malformations. Uteri without grossly visible implantation sites were stained by aluminium sulphide according to the procedure of Salewski, (1964) to identify the presence of early resorption sites while rats with no stained implantation sites were considered not pregnant.

Statistical Analysis

Data obtained from this study were expressed as the mean value ± standard error of mean. The data were analysed using one way analysis of variance (ANOVA) and differences between means of control and treated groups were determined using Statistical Package for Social Scientist (SPSS 11.0). p values less than 0.05 or 0.01 were considered statistically significant.

RESULTS

Effect of the extract on maternal body weights (GD 6-21).

The effect of the methanolic extract of the root of *Cissampelos mucronata* on mean maternal body weights administered from Gestation day 6 to Gestation day 20 are presented in Tables 1. The administration of the extract from gestation day 6 was observed to have caused a significant ($p<0.05$-0.01) decrease in body weight gain in the rats that were administered with 100 and 200 mgkg^{-1} of the extract when compared with the control (0 mgkg^{-1}) group that had a significant($p<0.05$-0.001) body weight increase however the effect was more severe in the group administered with 300 mgkg^{-1} of the extract which presented with a significant body weight loss due to the fact that their pregnancy status did not advance beyond gestation day 6 (Table 2).

Effect of the extract during GD 6-20 on caesarean section and foetal parameters

The effect of the administration of the extract on caesarean section and foetal parameters are presented on Table 2 and 3. The results indicate that administration of the extract from gestation day 6 to 20 had no significant effect on the number of implantation sites counted. Resorptions sites were however significantly ($p<0.05$-0.001) high following the administration of 100, 200 and 300 $mgkg^{-1}$ of the extract with the 300 $mgkg^{-1}$ dosed group showing a 100% resorptions due to the failure of the pregnancies in this group to progress beyond gestation day 6. Dead foetuses were completely absent in the 300 $mgkg^{-1}$ dosed group and significantly ($p<0.05$-0.01) high in the 100 and 200 $mgkg^{-1}$ dosed groups when compared with the control group while viable foetuses were completely absent in the 300 $mgkg^{-1}$ dosed group and significantly ($p<0.05$-0.01) low in the 100 and 200 $mgkg^{-1}$ dosed group. The number of males and females counted among the viable and dead foetuses were not significantly different from each other.

There was a decrease in the foetal weight, placental weight and crown rump length of the rats dosed with 100 and 200 $mgkg^{-1}$ of the extract with significance ($p<0.05$-0.01) observed in foetal weights and crown rump length (Table 3).

Table 1: Effect of the administration of the methanolic extract of the root of *Cissampelos mucronata* during GD 6-21 on mean maternal body weights.

		Maternal weights (g)			Body weight differences	
Doses ($mgkg^{-1}$)	**N**	**GD 0**	**GD 6**	**GD 21**	**GD0-GD6**	**GD6-GD21**
0	10	149.48±9.57	161.90±9.26	203.50±8.17^{aa}	12.42±2.58	41.61±2.14
100	10	143.41±7.79	157.85±8.21	187.11±7.72^{a}	14.44±2.66	29.25±2.74*
200	10	153.19±9.13	166.95±8.64	190.26±8.18	13.76±2.85	23.31±3.04**
300	09	162.86±9.92	180.48±11.49	172.40±9.43	17.62±6.13	-8.09±5.58**

Results are presented as Means ± SEM. Significance relative to body weight on corresponding gestation day **p<0.01,*p<0.05, **N**=Number of rats per group and **GD**= Gestation Day.

Table 2: Effect of the administration of the methanolic extract of the root of *Cissampelos mucronata* during GD 6-20 on caesarean section parameters .

	Doses administered($mgkg^{-1}$)			
Parameter (g)	**0**	**100**	**200**	**300**
Number of Females Mated	10	10	10	09
No. Pregnant (%)	10 (100)	10 (100)	10 (100)	9 (100)
No. Litters with Viable Foetuses	10 (100)	10 (100)	10 (100)	9 (100)

No. of implantation sites	12.00 ± 0.33	12.50 ± 0.40	11.70 ± 0.45	11.44 ± 0.42
Resorptions	0.00 ± 0.00	2.10 ± 0.28**	3.00 ± 0.39**	12.56 ±0.56**
Mean No. of Dead Foetuses per female	0.00 ± 0.00	0.70 ± 0.30*	1.10 ± 0.23**	___a
Total No. of Dead Foetuses per litter	00.00	07.00	11.00	___a
Males per litter (n)	00	04	04	___a
Females per litter (n)	00	03	07	___a

Significance relative to control (0 $mgkg^{-1}$) **p<0.01,*p<0.05

[a] Caesarean section parameters were not determined on non pregnant females.

Results are presented as Means ± SEM

Table 3 : Effect of the administration of the methanolic extract of the root of *Cissampelos mucronata* during GD 6-20 on foetal parameters .

	Doses administered($mgkg^{-1}$)			
Parameter (g)	**0**	**100**	**200**	**300**
Male : Female % ratio of Dead Foetuses	00	57:43	36:64	___a
Mean No. of Viable Foetuses per female	8.50 ± 0.45	6.50 ± 0.50**	5.20 ± 0.25**	___a
Total No. of Viable Foetuses per litter	85.00	65.00	52.00	___a
Males per litter (n)	46.00	35.00	24.00	___a
Females per litter (n)	39.00	30.00	28.00	___a
Male : Female % ratio of Viable Foetuses	54:46	54:46	46:54	___a
Foetal weight(g)	3.55 ± 0.24	2.64 ± 0.12**	2.10 ± 0.22**	___a
Placental weight(g)	0.72 ± 0.14	0.58 ± 0.02	0.54 ± 0.03	___a
Crown rump length(cm)	3.50 ± 0.08	2.87 ± 0.11**	2.20 ± 0.12**	___a

Significance relative to control (0 $mgkg^{-1}$) **p<0.01,*p<0.05

[a] Caesarean section parameters were not determined on non pregnant females.

Results are presented as Means ± SEM

DISCUSSION

Administration of the extract of *Cissampelos mucronata* from gestation day 6 to gestation day 20 slowed down body weight increase in the treated rats. This suggests that exposure to the aqueous extract of *Cissampelos mucronata* during pregnancy decreases pregnancy weight gain due to the loss of appetite observed during the course of the study as a result of the effect of the phytochemical constituents of the plant (Tsuda *et al.,* 2005 and Yu *et al.,* 2006).

The dose dependent and significant increase in the number of resorption sites in rats treated with 100 and 200 $mgkg^{-1}$ of the extract and 100% resorption in rats treated with 300 $mgkg^{-1}$ of the extract as shown in Table 2 and the number of dead foetuses per female rat which significantly increased with dose with the concominant low foetal weights and decreased crown rump length are all characteristic of the toxic effect of *Cissampelos mucronata* extract . The death of an embryo after implantation is defined as resorption - which is characterized as 'in situ' lyses of embryos or fetuses (Kalter, 1980). There was a significantly higher proportion of resorptions in *Cissampelos mucronata* extract treated mothers; hence it was possible to assume that *Cissampelos mucronata* extract was toxic to the embryos, causing its death after implantation. The toxic effect observed in this study might be attributed to the saponin contents of the extract because the negative effects of saponins on animal reproduction have been ascribed to their abortifacient, antizygotic and anti-implantation properties (Tewary *et al.,* 1973; Stolzenberg and Parkhurst, 1976). Saponins from broom weed or commercial pharmaceutical grade saponins have been shown to cause abortion or death or both in rabbits, goats and cows when administered intravenously at concentrations above 2·3 mg/kg body weight (Dollahite *et al.,*1962) with the ones isolated from the crude extract of *Gleditischia horrida, Costus speciosus* and *Phytolacca dodecandra* linked to sterility in mice (Chou *et al.,*1971;Tewary *et al.,* 1973; Stolzenberg and Parkhurst, 1976) while Quin and Xu (1998) found that the butanol extract of *Mussaenda pubescens* was capable of terminating pregnancy in rats. This toxic effect was confirmed by the lower body weight of fetuses and placentae in *Cissampelos mucronata* extract treated mothers. Normal fetal and organs growth and development are determined by a complex interplay between genetic, immunological, endocrinological, nutritional, vascular and environmental influences and a disturbance in one of these factors would disrupt normal growth and development (Chahoud *et al.,*1999).It is known that when a toxic agent causes death,or fetal growth delay without causing malformations, it can be related to the fact that it interferes with vital processes such as glycolysis, mitochondria function or membrane integrity.

According to Holemans *et al.* 2003 and Ergaz *et al.* 2005 reduction in fetal body weight could be related with nutrition, maternal metabolism and placental vascular alterations. Vascular placental pathology is a significant contributor for reduced growth in the intrauterine life (Ergaz *et al.,* 2005). Placentae from *Cissampelos mucronata* extract treated animals were significantly smaller than those from control group, suggesting some alterations in these organs but as no histopathological analysis was performed, it was impossible to elucidate the cause of this weight reduction.

In conclusion, our findings indicated that administration of the extract from gestation day 6 to 20 caused resorptions in pregnant and decrease in foetal weight, placental weight and crown rump length in rats, in a dose dependent manner. We recommend that further studies aimed at corroborating these findings be carried out.

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REFERENCES

1. Adjanohoun EJ, Ahyi MRA, Chibon P. 1989. In: Neuwinger H.D. 2000. African traditional medicine: A dictionary of plant use and applications. Medpharm Scientific Publishers, Stuttgart. Pp 231-236
2. Baerts M, Lehmann J. 1989. In: Neuwinger H.D. 2000. African traditional medicine: A dictionary of plant use and applications. Medpharm Scientific Publishers, Stuttgart. Pp 32-34
3. Burkill HM. 1997. The useful plants of West Tropical Africa vol 4. Royal Botanical Garden kew. Pp. 136-154.
4. Chahoud I, Ligensa, A, Dietzel L, Faqi A S. 1999. Correlation between maternal toxicity and embryo/fetal effects. Reprod.Toxicol., 13, 375-381.
5. Chhabra SC, Mahunnah RLA, Mshiu EN. 1990. Plants used in traditional medicine in eastern Tanzania. III. Angiosperms (Euphorbiaceae to Menispermaceae). Journal of Ethnopharmacology 28: 255-283
6. Chou SC, Ramanathan S, Matsui A, Rojers J, Cutting WC . 1971 . Isolation of saponins with antifertility activity from *Gleditschia horrida*. Indian Journal of Experimental Biology 9, 503–504.
7. Declaration of Helsinki. 1996. Amended by World Medical Assembly, Venice, Italy, 1983. Br. Med. J. 313(7070), 1448-1449
8. Dollahite JW, Shaver T, Camp BJ. 1962. Injected saponins as abortifacients. American Journal of Veterinary Research 23, 1261–1263.
9. Dunlap WP, Duffy JA. 1975. Fortran IV functions for calculating exact probabilities associated with Z, chi-square, T and F values. Behav.Res. Methods Instrum. 7, 59–60.
10. Elujoba AA. 1995. Female infertility in the hands of traditional birth attendants in south-western Nigeria. Fitoterapia 66: 239-248.
11. Ergaz Z, Avgil M, Ornoy A. 2005. Intrauterine growth restriction - etiology and consequences: What do we know about the human situation and experimental animal models? Reprod. Toxicol., 20,301-322.
12. Gelfand M, Mavi S, Drummond R.B. and Ndemera, B. 1985. The traditional medical practitioner in Zimbabwe. His principles of practice and pharmacopoeia. Mambo Press, Gwere. pp. 77–85
13. Holemans K, Aerts L, Van Assche FA. 2003. Fetal growth restriction and consequences for the offspring in animal models. J. Soc. Gynecol. Investig., 10, 392-399.
14. ICH. 2005. International Conference on Harmonisation (ICH) Guideline Harmonised Tripartite Guideline for the Detection of Toxicity to Reproduction to Medicinal Products and Toxicity to Male Fertility S5 (R2) pp. 3-8
15. Kalter H. 1980. The relationship between congenital malformations and prenatal mortality in experimental animals. In: Potter, I. and Hook, E. B. (Eds.). Human Embryonic and Fetal Death. New York: Academic Press. pp. 29-44.
16. Kokwaro JO. 1976. Medicinal plants of east Africa. East African Literature Bureau, Nairobi. . pp. 211-219.
17. Nwosu. MO. 1999. Herbs for mental disorders. Fitoterapia 70: 58-63.
18. Quin GW, Xu RS. 1998. Recent advances in bioactive natural products from Chinese medicinal plants. Medical Research Reviews 18, 375–382.

19. Salewski VE. 1964. Faerbermethode zum Makroskopischen Nachweis von Implantations Stellen am Uterus der Ratte. Naunyn-Schmeidebergs Archiv Pharmakol. Exper. Pathol. 247:367.
20. Stolzenberg SJ, Parkhurst RM. 1976. Blastocidal and contraceptive actions by an extract and compounds from endod (*Phytolacca dodecandra*). Contraception 14, 39–51.
21. Tewary PV, Chaturvedi C, Pandey VB .1973 . Antifertility activity of *Costus speciosus* Sm. Indian Journal of Pharmacology 35, 114–115.
22. Tshibangu JN, Chifundera K, Kaminsky R, Wright AD, König GM. 2002. Screening of African medicinal plants for antimicrobial and enzyme inhibitory activity. Journal of Ethnopharmacology 80: 25-35.
23. Tsuda T, Ueno Y, Kojo H, Yoshikawa T, Osawa T. 2005. Gene expression profile of isolated rat adipocytes treated with anthocyanins. Biochim Biophysica Acta 1733:137–47.
24. U.S.EPA, 1998. Office of Prevention, Pesticides and Toxic Substances (OPPTS), United States Environmental Protection Agency Health Effect Test Guidelines, OPPTS 870.3700, Prenatal Developmental Toxicity Study pp22
25. U.S.FDA, 2000. "Redbook 2000" Toxicological Principles for the Safety Assessment of Food Ingredients: Guidelines for Developmental Toxicity Studies. United States Food and Drug Administration. Centre for Safety and Applied Nutrition, Washington, DC.pp23
26. Van Wyk BE, Gericke N. 2000. People's plants. A guide to useful plants of Southern Africa. Briza Publications, Pretoria.
27. Watson L, Dallwitz MJ. 1992. The families of flowering plants: Descriptions, Illustrations,Identification and Information Retrieval. Available at: http:// biodiversity.uno.edu/ delta/angio/ www/ menisper.htm Accessed: November 2006
28. WHO 1993. Research Guidelines for the Evaluating the Safety and Efficacy of Herbal Medicines. Manilla, World Health Organization. Regional Office for the Western Pacific.pp31
29. WHO. 1998. Quality Control Methods for Medicinal Plant Materials. World Health Organization. Geneva.pp212
30. Yu SF, Shun CT, Chen TM, Chen YH. 2006. 3-O-beta-D-glucosyl-(1–N6)-beta-D-glucosyl-kaempferol isolated from *Sauropus androgenus* reduces body weight gain in Wistar rats. Biol Pharm Bull 29:2510–2513.