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Effect of Aqueous Extract of *Anacardium occidentale* Stem Bark on Maternal Hormones and Fetal Outcome in Pregnant Rats

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Abstract: This study was carried out to investigate the effect of aqueous extract of *Anacardium occidentale* stem bark on maternal hormonal levels and fetal outcome in pregnant rats. The animals were divided into four groups. The control group received a daily dose of distilled water and 100mg/kg of extract was administered to the other three groups during the 1st trimester (Day 1-7), 2nd trimester (Day 1-14), and 3rd trimester (Day 1-21) respectively. Progesterone, Luteinizing Hormone (LH) and Follicle-stimulating Hormone (FSH) were assayed for in the dams as well as implantation, gestation length and weekly maternal weights were determined. Litter sizes, fetal weights, placental weights were also recorded. The extract decreased the concentrations of serum progesterone in the Day 1-21 exposed dams. The FSH level was also significantly decreased in animals administered extract in the Day 1-14 and Day 1-21 while it was increased in animals in the Day 1-7 group. The level of LH was decreased in the Day 1-21 when compared with the control. The average weight of dams was significantly reduced throughout pregnancy, while the gestation length was not affected. The litter size, fetal weights and placental weights were significantly reduced in treatment groups compared to the control. The results suggest that the aqueous extract of *Anacardium occidentale* stem bark decreased hormonal levels resulting in lower pregnancy rates which could be the reason for the decrease in litter size and foetal weights.

KEYWORDS: *Anacardium occidentale*, fetal outcome, maternal hormones, trimesters

1.0 Introduction

Anacardium occidentale L., commonly referred to as cashew originated from Asia, Central America and Africa (Santos *et al.*, 2007). The medicinal parts of the plant include the nut, leaves, cashew oil as well as the stem bark (Thomson, 2004). The seed contain alkyl phenols, anacardic acid, cardol and methyl cardol, fatty acids as well as proteins (Thomson, 2004). In traditional medicine, *Anacardium occidentale* L. has been used to manage fever, pain, swellings (Thomson, 2004; Rajesh *et al.*, 2009), asthma (Olajide *et al.*, 2004), and also in the control of arthritis and other inflammatory conditions (Ojewole, 2004). The cashew stem bark possesses pain-relieving effects (Almeida *et al.*, 2000), anti-diabetic (Olatunji *et al.*, 2005) aphrodisiac and anti-malarial properties. In traditional medicine the cashew stem bark given to pregnant women as a pain reliever and as an anti-malarial.

This study was carried out to investigate the effect of aqueous extract of *Anacardium occidentale* stem bark on maternal hormonal levels and fetal outcome in pregnant rats

2.0 Materials and Methods

2.1 Plant material

The *Anacardium* stem bark was obtained from and identified at the University of Ilorin, Nigeria.

2.2 Assay kits

The assay kits for Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), and Progesterone were obtained from Roche Diagnostic GmbH (Mannheim, Germany).

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2.3 Preparation of extract

The powdered material was stocked in glassware from which 100 g was extracted in 1L of cold distilled water for 48 hours at room temperature. This was then filtered with Whatman No. 1 filter paper. The filtrate was concentrated on a steam bath to give 24.7 g of the residue (brownish black slurry). The residue was then reconstituted in distilled water to give the required dose of 100 mg/kg body weight that was used in this study.

2.4 Phytochemical screening

The aqueous extract of the *Anacardium occidentale* stem was screened for the presence of phenols, flavonoids, alkaloids, saponins, chalcones, terpenes, anthraquinones. Also quantitative determination was carried out (Van-Burden and Robinson, 1981; Harborne, 1984; El-Olemy *et al*., 1994; Obadoni and Ochuko 2001).

2.5 Animals and treatment

Male (300 ± 10.0g) and female (200 ± 10.0g) albino rats were housed at the animal house of the Physiology Department, University of Ilorin with a 12-h light: 12-h dark cycle and humidity: 50–55%. They were acclimatized for two weeks to the housing conditions and animals were allowed free access to rat pellets (Bendel Feeds and Flour Mill, Ewu, Nigeria) and tap water. The cages were cleaned daily. All animal handling and experimental protocols adopted in this study complied with the international principles for laboratory animals as obtained in the Helsinki's Declaration (NIH, 1985).

Virgin female rats in proestrus were mated overnight with males while successful mating was indicated by the presence of spermatozoa in the vaginal smear the following morning, day 1 of pregnancy, (Mallie and Boudzoumou, 1996). Twenty pregnant rats were randomly divided into four groups. The control group was administered a daily dose of distilled water as the vehicle, and 100mg/kg was administered to the other three groups during the 1st trimester (Day1-7), 2nd trimester (Day1-14), and 3rd trimester (Day 1-21) respectively. Dams were weighed weekly during pregnancy.

2.6 Collection of blood and determination of foetal and maternal indices

On day 21 of gestation, dams were killed with diethyl ether by inhalation. Blood was obtained by cardiac puncture for hormonal analysis.

The ovaries of dams were dissected, and the number of corpora lutea counted. The two-horned uterus was removed and examined for implantation sites (Cavieres *et al*., 2002). Gestation length was also observed and fetuses were individually weighed and examined for external malformations. The placentas were also weighed. Live foetuses were examined for any external deformities, thus fetal viability and resorptions were taken into account.

2.7 Statistical Analysis

The results were expressed as values of mean ± Standard error of mean (SEM). Means were analyzed using a one-way ANOVA and values of $p<0.05$ were considered statistically significant.

3.0 Results

The preliminary phytochemical screening of the aqueous extract of aqueous extract of *Anacardium occidentalis* stem bark revealed the presence of bioactive agents like alkaloids, phenols, saponins, flavonoids while terpenes, anthraquinones, and chalcones were not detected (Table 1). Quantitative analysis of the phytochemicals indicated that the aqueous extract of the stem of *Anacardium occidentalis* consisted largely of saponins, while the flavonoids were the least (Table 1).

Clinical toxicity symptoms such as respiratory distress, salivation, dull eyes, diarrhea and change in the appearance of fur were not observed in the animals although there was reduction in weight of the animals treated during the second trimester of pregnancy. The aqueous extract of *Anacardium occidentalis* stem bark exposure had effects on the three trimesters of pregnancy. The average weight of dams was significantly reduced in the 1st, 2nd and 3rd trimester groups on day 7 , day 14 and day

21 (Table 2) although the gestation length was compared with the control. There was also no case of mortality recorded.

The extract decreased the concentrations of serum progesterone in the dams exposed to the extract from day 1-21(third trimester), while follicle-stimulating hormone level was significantly decreased in animals administered extract from Day 1-14 and day 1 -21 (second and third trimesters respectively) while it was increased in animals in exposed to the extract on day 1-7 (first trimester). The level of luteinizing hormone was increased in the first and second trimester groups and decreased in the third trimester when compared with the control.

All the litters were delivered within a few hours after their normal gestation period. The litter size was significantly reduced in the Day 1-7, Day 1-14, and Day 1-21 groups when compared with the control. Placental weight was significantly reduced in rats exposed to the extract in the third trimester. Fetal weights were also significantly reduced in rats exposed to the extract in the third trimester when compared with the control ($P<0.05$). The body weights of the dams were reduced during the three trimesters whereas the percentage resorption of rats exposed to the extract increased in a manner dependent on the trimesters.

4.0 Discussion

According to authors like Ema and Harazono (2001), maternal body weight is now a sensitive indicator of toxicity after exposure to a substance. This was also seen in this study as weights in the pregnant rats were significantly reduced at Days 7, 14 and 21 during the treatment period. The litter sizes from the D1-7, D1-14 and D1-21 exposed dams were reduced as well as the foetal weights which is a manifestation of developmental fetotoxicity (Cavieres *et al*., 2002). Foetal resorption could be due to modifications of uterine lining function or maternal toxicity which consequently may increase early resorption and late foetal death (Chaves *et al*., 1985; Khera, 1987). Foetal weight correlates positively with placental weight (Heasman *et al*., 1999) thus the reduction in foetal weight in third trimester animals correlates to the decrease in placental weight observed in the study.

Table 1: Phytochemical constituent of *Anacardium occidentale* sterm bark

Phytochemicals	**Concentration (mg/L)**
Tannins/ Phenols	0.119± 0.01
Flavonoids	0.109 ±0.01
Alkaloids	0.085 ± 0.01
Saponins	0.124 ± 0.01
Chalcones	Not detected
Terpenes	Not detected
Anthraquinones	Not detected

n= mean ±SD of three determinations

Table 2: Effect of aqueous extract of *Anacardium occidentale* stem bark on fetal outcomes

	Control	(Days 1-7)	(Days 1-14)	(Days 1-21)
Dams				
No. of mated females	10	10	10	10
No. of pregnant dams	10	10	10	10
Dams' body weight (g)				
At Day 1	151.03± 6.74	153.48 ± 5.56	141.13 ± 4.99	139.50 ±3.00
At Day 7	162.65 ± 8.35	161.58 ± 6.09	137.90 ± 4.15*	137.28 ± 9.04*
At Day `14	167.08 ± 5.92	162.97 ± 6.19*	137.90 ± 4.16*	143.23 ± 9.22*
At Day 21	171.1 ± 5.47	165.97 ± 6.44*	135.50 ± 4.64*	145.73 ± 8.97*
Gestation Length	21.00 ± 0.51	21.00 ± 0.65	22.00 ± 0.50	22.00 ± 0.50
Implantation Sites	6.40 ± 0.45	6.3 ± 0.37	6.30 ± 0.53	5.9 ± 0.50
Fetuses				
Litter size	14.40 ± 0.99	12.27 ± 0.54*	13.50 ± 0.60*	11.50 ±0.75*
% Fetal Viability	100	79.9	64.8	40.00
% Fetal Resorption	0.00	20.10	35.2	60.00
Placental weight (g)	0.52 ± 0.03	0.50 ± 0.02	0.50 ± 0.07	0.30 ± 0.09*
Fetal weight (g)	6.2 ± 0.47	5.50 ± 0.34	4.50 ± 0.25	2.33 ± 0.39*

*Significantly different from control (p _ 0.05); **(p _ 0.005). Two-way ANOVA followed by t-test. Numbers are means ± SEM.

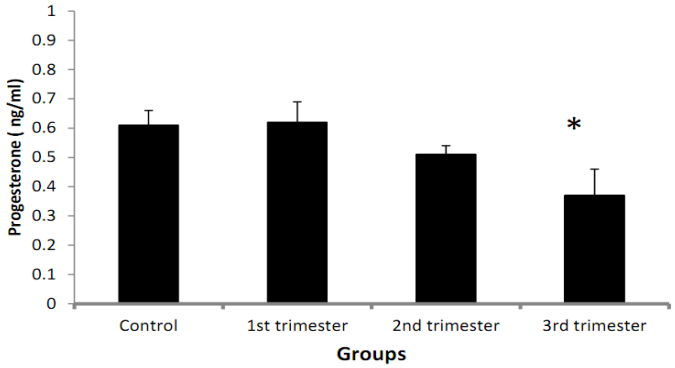

Figure 1: Effect of administering aqueous extract of *Anacardium occidentale* stem bark on progesterone level of the pregnant rats

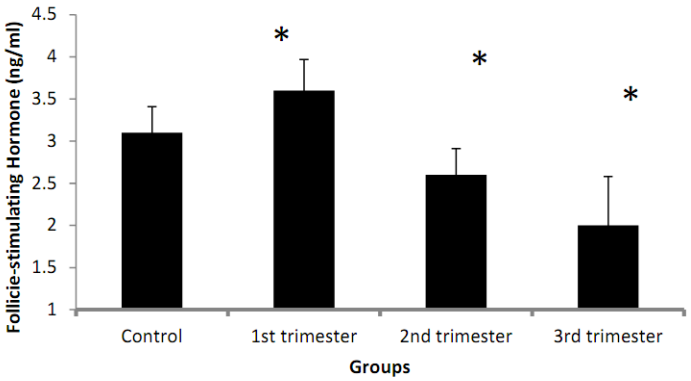

Figure 2: Effect of administering aqueous extract of *Anacardium occidentale* stem bark on Follicle- stimulating Hormone level of the pregnant rats

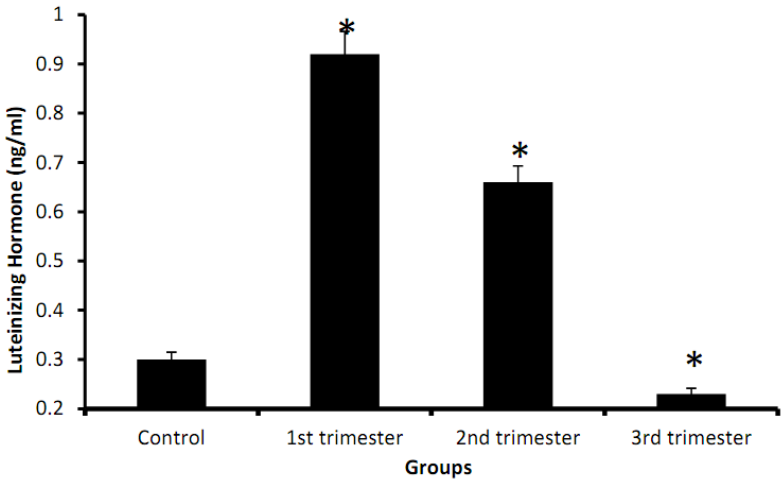

Figure 3: Effect of administering aqueous extract of *Anacardium occidentale* stem bark on Luteinizing Hormone level of the pregnant rats

This infers that placental weight correlates with maternal exposure to substances, this is because substances cross through the mother to the fetus. Thus the extract was not any different. Matthews et al., (2004) explain that maternal exposure to some substances till the later part of pregnancy result in low fetal as well as placental weights and the report is consistent with the present study.

Appropriate determination of hormonal levels has been used to diagnose the state of pregnancy. In the present study hormones such as progesterone, FSH were analyzed as a result of their roles in pregnancy to detect an ectopic or failing pregnancy (Radwanska *et al.*, 1978). The reduction in the concentration of FSH in the animals exposed at day 1-14 and day 1-21 is an indication of impairment in the reproductive cycle of the animals (Simoni and Nieschlag, 1995). LH is required for continued development and normal functioning of the corpora lutea. The significant reduction in the level of serum LH in the animals exposed during day 1-21 could be associated with the physiological process of luteolysis preceding parturition (Taya and Greenwald, 1981). It could possibly be attributed to hormonal imbalance resulting from a luteal phase that is not being maintained. The reduced level of the hormone may also be due to inactivation of ovarian follicles (Morishige *et al*., 1973), which could be responsible for the reduction in the concentration of serum progesterone in this study. Progesterone, which is elevated during pregnancy, plays a major role in maintaining the condition and is an important factor in the implantation process during pregnancy. Thus, luteolysis and the decrease in the blood levels of progesterone may contribute to high resorption observed in the day 1-21 treatment group exposed of the *Anacardium occidentale* stem bark extract in this study.

Although *Anacardium occidentale* stem bark has been accrued many beneficial uses in the treatment of diseases, caution should be excised when consuming it during pregnancy.

References

Almeida, R. N., Almeida, R. N., Navarro, D. S. and Barbosa-Filho, J. M. (2000). Plants with central analgesic activity. Phytomedicine 8: 310-322.

Cavieres, F. M., Jaeger, J. and Porter, W. (2002). Developmental toxicity of a commercial herbicide mixture in mice: Effects on embryo implantation and litter size. Environmental Health Perspective 110(11): 1081-1085.

Chaves, D. J. and Anderson, T. L. (1985). The glycocalyx of the mouse uterine luminal epithelium during estrus, early pregnancy, the pre-implantation period, and delayed implantation: I. Acquisition of *Ricinus communis* I binding sites during pregnancy. Biology and Reproduction 32:1135–1142.

El-Olemy, M. M, Al-Muhtadi, F. J. and Afifi, A. A. (1994). Experimental phytochemistry. A laboratory manual. Riyadh, Saudi Arabia: King Saud University Press.

Ena, M. and Harazono, A. (2001). Toxic effects of butyltin chloride during early pregnancy in rats. Toxicology Letters 125(1-3): 99-106.

Harborne, J. B. (1984). Phytochemical methods. A guide to modern techniques of plant analysis. London: Chapman and Hall.

Heasman, L., Clarke, L., Stephenson, T. J. and Symonds, M. E. (1999). The influence of maternal nutrient restriction in early to mid-pregnancy on placental and fetal development in sheep. Proceedings of the Nutrition Society 58, 283-288.

Khera, K. S. (1987). Maternal toxicity in humans and animals: effect on fetal development and criteria for detection. Teratogenic Carcinogenic Mutants 7:287-295.

Mallie, J. P. and Boudzoumou, P. (1996). Functional renal maturation in rats neonates after prenatal exposure to furosemide. Pediatric Nephrology 10: 458-460.

Matthews, F., Youngman, L. and Neil, A. (2004). Maternal circulating nutrient concentrations in pregnancy: implications for birth and placental weights of term infants. American Journal of Clinical Nutrition 79:103-110.

Morishige, W. K, Pepe, G. J and Rothchild, I. (1973). Serum luteinizing hormone, prolactin and progesterone levels during pregnancy in the rat. Endocrinology 92:1527–1530.

NIH. (1985). Guide for the Use of Laboratory Animals DHHS, PHS.NIH Publication No. 85-23.

Obadoni, B. O. and Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Science 8:203-208.

Ojewole, J. A. (2004). Potentiation of the anti-inflammatory effect of *Anacardium occidentale* (Linn.) stem-bark aqueous extract by grapefruit juice. Methods and Findings in Experimental and Clinical Pharmacology 26: 183-188.

Olajide, O. A., Aderogba, M. A., Adedapo, A. D. A. and Makinde, J. M. (2004). Effects of *Anacardium occidentale* stem bark extract on *in vivo* inflammatory models. Journal of Ethnopharmacology 95: 139-142.

Olatunji, L. A., Okwusidi, J. I. and Soladoye, A. O. (2005). Antidiabetic effect of *Anacardium occidentale* stem-bark in fructose-induced diabetic rats. Pharmaceutical Biology 43(7): 589-593.

Radwanska, E., Frankenberg, J. and Allen, E. (1978). Plasma progesterone levels in normal and abnormal early human pregnancy. Fertility and Sterility 30:398-402.

Rajesh, K.V., Sumathi C. S., Balasubramanian, V. and Ramesh, N. (2009). Elementary chemical profiling and antifungal properties of Cashew (*Anacardium occidentale* L.) nuts. Botanical Research International *2*: 253-257.

Santos, R. P, Santiago, A. A. X., Gadelha, C. A. A., Cajazeiras, J. B., Cavada, B. S., Martins, J. L., Oliveira, T. M., Bezerra, G. A., Santos, R. P. and Freire, V. N. (2007). Production and characterization of the cashew (*Anacardium occidentale* L.) peduncle bagasse ashes. Journal of Food Engineering 79: 1432-1437.

Simoni, M. and Nieschlag, E. (1995). FSH in therapy: physiological basis, new preparations and clinical use. Reproductive Medicine Review 4:163–177.

Taya, K. and Greenwald, G. S. (1981). *In vivo* and *in vitro* ovarian steroidogenesis in the pregnant rat. Biology and Reproduction 25:683-691.

Thomson, P. D. R. (2004). PDR for herbal medicines. 3rd edition Thomson PDR, New Jersey, U.S.A., pp. 165-166.

Van-Burden, T. P. and Robinson, W. C. (1981). Formation of complexes between protein and tannic acid. Journal of Agriculture and Food Chemistry1:77-82.