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Effects of Aqueous Extract of *Anacardium occidentale* (Cashew) Leaf on Pregnancy Outcome of Wistar Rats

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Abstract: *Anacardium occidentale* (cashew) leaf extract is used medicinally to treat various kinds of diseases such as diabetes, fever, bronchitis etc, in different parts of the world including Nigeria. This study investigates the effect of *A. occidentale* leaf extract on reproductive outcome of Wistar rats. Twenty female wistar rats were divided into 4 groups (I, II, III, and IV), n = 5 in each group. Group I is the control while groups II, III and IV are experimental groups. 300 mg/kg of aqueous leaf extract of *A. occidentale* was administered orally to group II, III and IV during early (days 1-7) mid- (days 7-14) and late (days 14-21) pregnancy respectively. The leaf extract significantly ($p<0.05$) shows low birthweight on the pupsborn in early and mid pregnancy but high birthweight in late pregnancy: (Control vs treatment; early pregnancy, 7.220±0.030 vs 5.753±0.166 g; mid pregnancy, 7.220±0.030 g vs 5.431±0.149 g; late pregnancy, 7.220±0.030 vs 7.60±0.00 g), Crown-rump length (control vs treatment: early pregnancy, 6.450±0.050 vs 5.875±0.139 g; mid pregnancy, 6.450±0.050 vs 5.713±0.064 g; late pregnancy, 6.450±0.050 vs 6.50±0.00 g), Head Circumference, (control vs treatment: early pregnancy, 1.150±0.05 vs 1.225±0.016 g; mid pregnancy, 1.150±0.05 vs 1.138±0.026 g; late pregnancy, 1.150±0.05 vs 1.30±0.00 g). Life birth index is unaltered. Quantal pregnancy (%) in groups I, II, III and IV is 75, 80, 80 and 75, respectively. Litter size in group I, II, III and IV is 2, 8, 8 and 1, respectively. Gestation length is unaltered. The leaf extract was non teratogenic. In conclusion, the leaf extract of *A. occidentale* administered revealed low birthweight, crown-rump length in early and mid pregnancy but increased birthweight and crown-rump length in late pregnancy.

Key words: *Anacardium occidentale*, birthweight, crown-rump length, pregnancy, teratogenic

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Eisner, 1990). The Cashew (*Anacardium occidentale*) is a tree in the family of the flowering plant *Anacardiaceae*. The family contains 73 genera and about 600 species. *Anacardium* contains 8 species, native to tropical America, of which the cashew is by far the most important economically. It is a multipurpose tree of the Amazon that grows up to 15m high. It has a thick and tortuous trunk with branches so winding that they frequently reach the ground (Morton, 1987). The cashew tree produces many resources and products. The bark and leaves are used medicinally. The cashew nut has international appeal and market value as food. Even the shell oil around the nut is used medicinally and has industrial applications in plastics and resin industries for its phenol content. The pseudo-fruit, a large pulpy and juicy part, have a fine sweet flavor and is commonly referred to as the "cashew fruit" or the "cashew apple".

Cashew leaf and bark tea is used in Brazil and Peruvian herbal medicine, Tikinia in northwest Amazona, Wayapi tribe of Guyana as a douche for vaginal discharge and common diarrhea remedy. In Brazil, it is also used to treat diabetes, weakness, muscular debility, urinary disorders, asthma, eczema, psoriasis, scrofula, dyspepsia, genital problems, bronchitis, cough, intestinal colic, leishmaniasis, venereal diseases, as well as impotence, and syphilis-related skin disorders. In sixteenth-century, Brazil cashew fruits and their juice were taken by Europeans to treat fever, sweeten breath, and "conserve the stomach". It is taken for syphilis and as a diuretic, stimulant and aphrodisiac. In addition to being delicious, cashew fruit is a rich source of vitamins, minerals, and other essential nutrients. It has up to five times vitamin C than oranges and contains a high amount of mineral salts. Because of its high amount of vitamin C and mineral salts, cashew fruit is used as a catalyst in the treatment of premature aging of the skin.

Several clinical studies have shown that anacardic acids a component of cashew, with highest concentration in the nutshells curb the darkening effect of aging by

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inhibiting tyrosinase activity, and that they are toxic to certain cancer cells (Kubo *et al*., 1994).

Toyomizu *et al*. (2003) reveal a unique function of anacardic acid in that, for dietary conditions enhancing body fat deposition, that is consumption of a diet high in carbohydrates, dietary anacardic acid has the potential to decrease body fat deposition.

The effect of the methanol leaf extract, dichloromethane, ethyl acetate and *n*-hexane fractions from *Anacardium occidentale* Linn was investigated in streptozotocin-induced diabetic rats. Oral administration of methanol extract at doses of 35, 175 and 250 mg kgG1 significantly reduced blood glucose levels in diabetic rats 3 h after administration (Sokeng *et al*., 2007).

Further studies have shown the anti-bacterial, antifungal and anti-tumor activities of Cashew Apple Juice (CAJ), a processed juice from the pseudofruit of the cashew tree (Kubo *et al*., 1993a, b; Kozubek *et al*., 2001) as well as anti-oxidant effects (MeloCavalcante *et al*., 2003) and anti-mutagenic activity (Santos *et al*., 2002; MeloCavalcante *et al*., 2003).

There are no reports in the literature on the effects of aqueous extract of *Anacardium occidentale* (cashew) leaf on pregnancy outcome of wistar rats. The Present study therefore was undertaken to evaluate the effect of this plant on pregnancy outcome of wistar rats.

MATERIALS AND METHODS

Plant material: Samples of the leaves of Cashew were collected within the main campus of Ahmadu Bello University in October, 2010. The plant was identified authenticated by M. Musa of the herbarium section in the Department of Biological Science, Ahmadu Bello University, Zaira.

Preparation of the extract: Fresh leaves of Cashew were collected, air dried at room temperature and pulverized with pestle and mortar. 200 g of the powder was macerated with 150 mL of distilled water. The extract obtained was concentrated and evaporated to dryness on a water bath at a temperature of 60ºC to obtain a brownish mass of 20 g.

Experimental animals: Twenty Adult Wistar rats of both sexes weighing 180-300 g were used for the experiment. They were kept under well ventilated condition, fed on standardfeed (Excel feeds PLC) and allowed water ad libitum.

Grouping and mating of animals: The weight of the female Wistar rats were measured using an electronic weighing balance called Soehle, obtained from department of Human Anatomy, Faculty of medicine A.B.U. Zaria.The rats were grouped into four (4) groups (I, II, III and IV) of 5 rats each, according to their weights. Male Wistar rats were obtained from the same laboratory and introduced into each group for mating. They were left in darkness overnight overnight (Akpaffiong *et al*., 1986).

Smearing of vaginal: On the following day after mating, the vaginal smear was taken from each female rats in each group and observed to confirm mating. The materials used were 5ml syringe, beaker, distilled water, glass slides, and light microscope. One ml distilled water was injected with a syringe into the vaginal of each rat after which the vaginal fluid was collected, smeared on the glass slides and then viewed under the light microscope. The presence of sperm cells in the vaginal fluid indicated or confirmed a positive sperm test thus a successful mating (Akpaffiong *et al*., 1986).

The date of mating confirmation was marked as day one of gestation and the administration of extract started immediately.

Administration of extract: Extract was administered orally to the rats by carefully inserting the syringe with needle affix into the oral cavity of the rats. Group I (control) were given distilled water while group II, III, and IV (experimental groups) were given the extract 300 mg/kg at day 1-7 (first trimester), day 7-14 (second trimester) and day 14-21 (third trimester), respectively. Throughout the period of administration, food and water were given to the rats. The weight of each pregnant rat for both control and experimental groups were measured at days 1, 8, and 15 of the gestation period. Cage side examination were performed daily to detect overt signs of toxicity (salivation, lacrimation, chewing jaw movements, ptosis, squinting, writhing, convulsion, tremors, yellowing of fur, loss of hair, stress, erection of fur, vocalization and exophthalmia, behavioural abnormalities and moribound or dead rats) (Ratnasooriya *et al*., 2003).

Parturition: After parturition, the numbers of viable or still born pups were recorded. All pups were evaluated for gross external congenital abnormalities (open eyelids, tail anomalies, club foot, oligodactyl or syndactyl). The birth weight, crown-rump length and head circumference of the pups were determined. The birth weight of the pups was measured using Electronic Top Loading (Xp - series weighning balance, obtained from department of pharmaceutics, faculty of pharmaceutical sciences A.B.U Samaru, Zaria. The crown-rump length and head circumference were measures using ruler and string.

Pups mortality up to 6 days, the days of eye opening and appearance of fur were observed. (Ratnasooriya *et al*., 2003). Based on these data, the following indices were computed:

i) Quantal pregnancy= $\frac{\text{No. of pregnant dams X 100}}{\text{No. Mated}}$

Table 1: Change in body weight of pregnant rats on days 1, 8 and 15 of the gestation period.

Gestation days	Group I (Control) N = 4	GroupII (expt) N = 5	GroupIII (expt) N = 5	GroupIV (expt) N = 4
Day 1	183.5±17.212	226.4±9.086	21.8±26.852	211.5±15.521
Day 8	194.5±4.773	232.8±11.236	229.2±31.879	231.5±10.595
Day15	196.5±14.660	244.0±10.450	230.4±33.772	239.5±16.008

ii) Life birth index = $\frac{\text{No. of liveborn pups X 100}}{\text{Total no. of pups born}}$

iii) Gestation length

iv) Litter size

Statistical analysis: Data are reported as Mean ± Standard Error of Mean (SEM). One way analysis of variance (ANOVA) was to be statistically used to compare the weight of pregnant Wistar rats in various groups on day 1, 8 and 15 of gestation period. Birth weight, head circumference and crown-rump length of litters in control and experimental groups were checked for significance difference using student t-test. $p<0.05$ was deemed statistically significant. Sigmastat 2.0 (Systat Inc, Point Richmond, CA) was used for the statistical analysis.

RESULTS

Physical and behavioral observations: From observations made, there was no difference in behavioral changes noticed between the control group (I) and experimental groups (II, III and IV). There was no mortality, treatment-related signs of maternal toxicity, stress or abnormal behavioral changes observed within the experimental groups throughout the gestation period. None of the pregnant rats showed vaginal bleeding or expulsion of products of conception. Overt signs of toxicity such as rhinorrhea, lacrimation, squinting and, exophthalmia etc. were not expressed by the rats throughout the gestation period (Ratnasooriya *et al*., 2003). Thus, the above observation shows that the administered extract was non-toxic at the administered dose.

Body weight: The body weight (grams) of the pregnant rats in control and experimental groups, measured on the first, eighth and fifteenth day of pregnancy (gestation period) shows variations. The mean body weight of the rats in control:

Group I: on the 1st, 8th and 15th days of gestation period are 183.5±17.212, 194.5±14.773 and 196.5±14.660, respectively.

Group II: (experimental), mean body weight of rats on 1st, 8th and 15th days of gestation period are 226.4±9.086, 232.8±11.236 and 244.0±10.450, respectively.

Group III: (experimental), mean body weight of rats on 1st, 8th and 15th days of gestation period are 210.8±26.852, 229.2±31.879 and 230.4±33.772, respectively.

Group IV: (experimental), mean body weight of rats on 1st, 8th and 15th days of gestation period are 211.5±15.521, 231.5±10.595 and 239.5±16.008, respectively.

In all the groups, both control and experimental, there were no statistically significant difference. Data concerning the changes in body weight of pregnant rats are presented in Table 1.

Gestation period: Gestation periods of the experimental rats were observed longer than the control which is normally 21 days.

In group II, average gestation period of the two rats that littered was 24 days.
In group III, gestation period was 22 days.
In group IV, gestation period was 25 days.

Gross morphometric observation on the pups born: There was no still-births and/or birth abnormalities observed in all the pups born. Some of the parameters determined on the pups born in the control and experimental group shows variations. These are:

Body weight of Pups born: The mean body weight of pups born in group I is 7.220±0.030, group II is 5.753±0.166, group III is 5.431±0.149 and group IV is 7.600±0.00.

Litter size: The litter size of group I was 2, while that of experimental groups, II, III and IV are 8, 8, 1, respectively.

Crum-rump length: The mean Crum-rump length (cm) of pups born in group I is 6.45±0.05 while that of the experimental groups; II, III and IV are 5.875±0.139, 5.713±0.0639 and 6.50±0.00, respectively.

Head circumference: The mean head circumference (cm) of group I (control) is 1.150±0.05, while that of the experimental groups; II, III and IV are 1.225±0.016, 1.138±0.026 and 1.30±0.00, respectively.

For all these parameters (Weight of pups born, Crum-rump length and Head circumference), there is a statistically significant differences in the mean values of the treated groups.

Table 2: Effects of aqueous extract of *Anacardium occidentale* leaves on some litter parameters

Parameters	Group I (Control) n = 5	GroupII (expt) n = 5	GroupIII (expt) n = 5	GroupIV (expt) n = 5
Mean birth weight (g)	7.220±0.030	5.753±0.166	5.431±0.149	7.60±0.00
Mean Crown-rump length (cm)	6.450±0.050	5.875±0.139	5.713±0.0639	6.50±0.00
Mean Head circumference (cm)	1.150±0.05	1.225±0.016	1.138±0.026	1.30±0.00
Life birth index (%)	100	100	100	100
Quantal pregnancy (%)	75	80	80	75
Litter size	2	8	8	1
Gestation length (days)	21	24	22	25

For Weight of pups born p = <0.001
Crum-rump length p = 0.022
Head circumference p = 0.037

Life birth index: All the groups are 100%
Quanta pregnancy: group I and IV are 75% each and, group II and III are 80% each.

v) Litter size in groups I, II, III and IV are 2, 8, 8, and 1, respectively.
vi) Gestation length for groups I, II, III and IV are 21, 24, 22 and 25, respectively.

Data concerning litters parameters are presented in Table 2.

DISCUSSION

Results obtained from weighing the pregnant rats both in control and experimental groups on the 1st, 8th and 15th day of pregnancy were subjected to statistical analysis to determine the mean values for each group. The differences in the mean values of the weight of treated rats were not great enough to exclude the possibility that the difference is due to random sampling variability. Hence there is not a statistically difference in all the groups. This could have resulted from the action of anacardic acid, a component of the extract which was found to reduce the deposition of fat by its uncoupling action (Toyomizu *et al*., 2003).

Considering the pups born, with low birth weight, it has been established that extract of *A. occidentale* has antidiabetic action resulting in alleviation of altered metabolic status in animals. However, the action by which the extract lowered blood glucose in not well known, it may increase glycogen level in liver by an increase in glycogenesis and/or a decrease in Glycogenolysis (Tedong *et al.*, 2006).

Phytochemical analysis had revealed the presence of alkaloids, polyphenols, saponins etc. and based on the increased number of reports on blood glucose retardation associated with some saponins (Dietewa *et al*., 2004) and alkaloids (Bolkent *et al*., 2000) isolated from other medicinal plants, it is likely that the active principles could be present in one or the two families of the chemical substances (Tedong *et al*., 2006). Also anacardic acid has been shown to have an uncoupling effect on oxidative phosphorylation in the rat liver mitochondria using succinate as a substrate and hence dietary anacardic acid revealed to have the potential to decrease body fat deposition (Toyomizu *et al*., 2003).

The pups born to rats/dams treated with leaf extract of *A. occidentale* has low mean birth weight and less crown-rump length in group II and III compared to group I (control). However mean birth weight and mean crown-rump length of the pups in group IV is greater than that of group I (control). Low mean birth weight and crown-rump length revealed in group II and II could be as a result of the action of saponins or alkaloids and anacardic acid component of the extract causing impaired glucose supply to the fetuses and leading to Intrauterine Growth Retardation (IUGR). This is evident from the report of Pond (1996) that some antidiabetic drugs induce IUGR.

Since there was no any birth anomalies observed, low birth weight observed here may be due to impaired glucose supply to the fetuses and probably not as a result or effect of intrinsic fetal factor such as chromosomal abnormalities or other malformation.

Higher mean birth weight, mean crown-rump length and head circumference observed in group IV compared to control group might have resulted due to inhibition of saponins, alkaloids and anacardic acid by hormones or enzymatic action occurring during this period. It could also be due to low litter size (one) that enhance the weight increase of the litter.

It is important to note that length and birth weight as reported by Eide *et al*. (2005), each contribute independently both to adult stature and body weight, even though birth weight influences adult weight. In past decades, growing acceptances of the "fetal origin" of adult disease hypothesis have been witnessed, which has now led to the thought that poor intrauterine growth is associated with risk of cardiovascular disease and Non Insulin Dependent Diabetes Mellitus (NIDDM) in adult life. Birth weight studies have also shown that birth weight is an important predictor of adult height with babies of Normal Birth Weight (NBW) growing taller than babies with low birth weight.

Since this present studies revealed the low birth weight and low crown-rump length effect of *A. occidentale* extract, it is therefore means that consumption of the leaf extract during pregnancy have serious implications. Han *et al*. (2000) reported that low birth weight has a major influence on neonatal morbidity, neurocognitive deficiencies, neurobehavioural effects and

mortality. Ravelli *et al*. (1998) also reported that reduced growth in utero to be linked to decrease glucose tolerance in adult life.

CONCLUSION

This investigation was carried out to see the effect of *A. occidentale* on pregnancy outcome of wistar rats. It was observed that there was no significant increase in bodyweight of pregnant rats during gestation period. Pups born in group II and III (experimental) has low mean birth weight and less mean crown-rump length compared to group I (control), while that of Group IV control is higher than control. In spite of the beneficial effects of *A. occidentale* in treatment of diseases, its adverse effect that goes alongside cannot be overrule and must therefore be taken care of. Thus from the observations made in this studies, I want to conclude that the extract of *A. occidentale* should not be taken by pregnant women, even when associated with diabetes, during pregnancy.

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AUTHOR'S CONTRIBUTION

Hamman W.O.: Major supervisor; S. Musa: Minor supervisor; A.D.T. Goji: Analysis of data; A.A . Oyewale: Assisted in the review of the paper; S. Abba and I. Ezekiel: Colleagues in laboratory work.