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EFFECTS OF MATERNAL CONSUMPTION OF AQUEOUS LEAF EXTRACT OF *AZADIRACHTA INDICA* ON POSTNATAL WEIGHT GAIN AND NEONATAL MORTALITY IN ALBINO WISTAR RATS

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ABSTRACT

The present study was designed to investigate the effect of consumption of aqueous extract of *Azadirachta indica* during pregnancy on postnatal weight gain and neonatal mortality. Twenty female rats weighing between 150-200 g were used. On confirmation of pregnancy the rats were randomly grouped into four groups, I-IV of 5 rats each. Group I was the control group and received distilled water only. Groups II-IV were the test groups and received 200 mg, 400mg and 600 mg/kg body weight of the extract respectively. Rats in all groups had normal rat chow and water *ad libitum*. On the day of delivery, litter size and litter weight were recorded. This continued at seven days interval until postnatal day 56. Also, the neonatal mortality was determined at postnatal day 7. There was no significant difference ($P>0.05$) in the litter size in all the test groups when compared with the control. There was significant decrease ($p<0.05$) in postnatal weight gain from day 0 to postnatal day 56 in all the test groups when compared with the control group. Groups II and III showed no significant decrease ($p>0.05$) in neonatal mortality when compared with the control group. However, a highly significant decrease ($p<0.01$) in neonatal mortality was

observed in group IV. It can be concluded that aqueous leaf extract of *A. indica* has the potential of reducing postnatal weight gain and increases neonatal mortality.

KEYWORDS: *Azadirachta indica*, neonatal mortality, birth weight, postnatal weight gain, pregnancy.

INTRODUCTION

Women remain susceptible to illnesses at any time during pregnancy and possibly require treatment with prescription drugs to protect their own health as well as that of the fetus. In other circumstances, a woman may be taking a medication before she is aware of her pregnancy, or become pregnant while undergoing a treatment. However, among all drug treatments approved, a good number of them still have undetermined teratogenic risks associated with human pregnancies (Lo and Friedman, 2002).

Azadirachta indica (*A.indica*), popularly known as neem belongs to the family of *meliaceae* (Yanpallewar *et al*, 2003, Von Maydell, 1986). It is a popular medicinal plant originally grown in India but now being cultivated in almost every part of the world including Nigeria (Das *et al*, 2003) where it is popularly called "*Dogonyaro*". The plant is one of the most studied and widely used medicinal plants of all ages (Nwachukwu *et al*, 2009, Kausik and Ranajet, 2002). The importance of the neem tree has been recognized by the US National Academy of Sciences, which published a report in 1992 entitled 'Neem - a tree for solving global problems'. The advancement of neem research has earlier been documented (Schmutterer 1995). More than 135 compounds have been isolated from different parts of *A.indica* and several reviews have also been published on the chemistry and structural diversity of these compounds (Koul *et al*, 1990, Chatterjee *et al*, 1994, Devakumar *et al*, 1996). Some of its phytochemical constituents like; tannins, saponins, alkaloids, flavonoids and polyphenols (Atangwho *et al*, 2009) are responsible for its various actions. It has been used locally for the treatment of malaria, digestive disorders, body heat, fever, wounds, painful periods and jaundice (Ekaluo *et al*, 2010) and as an herbal pesticide (Okereke, 2003; Obi, 2004). Aqueous leaf extract of *A.indica* has been shown to demonstrate hypoglycemic and antidiabetic (Gupta *et al*, 2008, Khosla *et al*, 2008), antimalarial (Khalid *et al*, 1989, Isah *et al*, 2003), antihypercholesteremic (Chattopadyay et al, 2000), antifertility (Despande *et al*, 1980, Sathiyaraj *et al*, 2010) and immunostimulatory (Ray *et al*, 1996, Sen *et al*, 1992) activities in various experimental animal models. A recent study has reported significant increase in some hematological parameters and decrease in blood glucose level in rats treated

with aqueous leaf extract of *A.indica* during pregnancy thus, ameliorating the burden of anemia and hyperglycemia during pregnancy (Iyare and Obaji, 2014). In Nigeria decoctions and aqueous leaf extract of *A.indica* are commonly used in the treatment of malaria (Ucheya *et al*, 2011). Some women have been observed consuming aqueous extract of *A.indica* during pregnancy because of the belief that it is potentially harmless, makes them feel lighter and protects them from malaria, since pregnancy is associated with malaria. There is however, paucity of data on the effects of consumption of *A. indica* during pregnancy on postnatal weight gain and neonatal mortality rate. The present study was therefore designed to investigate the effect of consumption of aqueous leaf extract of *A.indica* during pregnancy on postnatal weight gain and neonatal mortality in albino rats.

MATERIALS AND METHODS

PLANT MATERIALS AND EXTRACT PREPARATION

Fresh matured leaves from the *A. indica* tree located at the University of Nigeria, Enugu campus were harvested and identified by Prof. M.O.Nwosu of Botany Department of the university where a voucher specimen was deposited at the herbarium for further reference (UNH No. 521^{A}). The leaves were then washed and air-dried. The dried leaves were homogenized using an electric blender. The powder was then exhaustively extracted in distilled water at 60^{o}c for 8 hours, using soxhlet extractor according to the method of Biu *et al*, (2010). The extract was then concentrated in a water bath (40^{o}c) yielding 340g of brown oily substance which was then stored at room temperature (27^{o}c) until use. The extract was reconstituted into 200, 400 and 600mg/kg body weight when needed.

EXPERIMENTAL ANIMALS AND EXTRACT ADMINISTRATION

Twenty mature inbred female, non-pregnant rats weighing between 150-200g were used for this study. The rats were housed in well ventilated cages and acclimatized for 3 weeks in the animal house of the Department of Physiology, University of Nigeria, Enugu campus under controlled environmental conditions. The animals were provided standard rat pellet feed and tap water *ad libitum*.

The estrus cycle was monitored for each rat by examining the daily vaginal smears under light microscopy and two consecutive regular four-day estrus cycles were noted. At pro-estrus, male rats of proven fertility were introduced into the female cages in the ratio of 1:2 to allow for mating. Mating was proved successful when spermatozoa were observed in the

vaginal smear of the female rats the following morning and this was regarded as day 1 of pregnancy (Mallie and Boudzoumou, 1996).

On day 1 of pregnancy, the rats were divided into four groups, labeled I- IV of 5 rats each. Group I rats served as the control group and received distilled water throughout the experiment while groups II – IV rats were the test groups and received 200mg/kg, 400 mg/kg and 600 mg/kg body weight of the extract respectively by gavage for 21 days.

On the day of delivery, the litter size delivered by each dam was recorded and the average determined. The average birth weight of the offspring delivered by each dam in each group on the day of delivery was determined using digital electronic weighing scale model no. LP505A made in China. This was recorded to the nearest (g). This continued at seven days interval until postnatal day 56.

The number of dead neonates within postnatal day 7 in each group was also determined and recorded.

All procedures used in this study were approved by institutional ethical committee and conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the Care and Use of animals (American Physiological Society, 2002).

STATISTICAL ANALYSIS

All data were tabulated and statistically analyzed using SPSS version 20.0. Results were expressed as Mean ± standard deviation (M ± SD). One way analysis of variance (ANOVA) followed by Bonferroni's Post-hoc test were used for data comparison. $P < 0.05$ was taken as statistically significant.

RESULTS

Table 1: Effects of aqueous leaf extract of *A.indica* on litter size, birth weight and neonatal mortality.

Groups	Litter size	Mean Birth weight (g)	Neonatal mortality
I	5.50±0.71	6.95±0.58	0.60±0.55
II	6.50±0.71	6.18±0.59*	0.80±0.45
III	7.50±0.71	5.65±0.31**	1.40±0.55
IV	6.00±0.01	4.77±0.20***	2.00±0.71**

Compared with the control *p<0.05, **p<0.01, ***p<0.001.

Aqueous leaf extract of *A.indica* showed no significant difference in litter size in all the test groups when compared with the control group. There was a significant decrease in birth weight in all the test groups when compared with the control group. However, this was in a dose dependent manner with the highest level of significance ($p<0.001$) at the concentration of 600mg/kg. Groups II and III showed no significant decrease ($p>0.05$) in neonatal mortality when compared with the control group. However, a highly significant decrease ($p<0.01$) in neonatal mortality was observed in group IV.

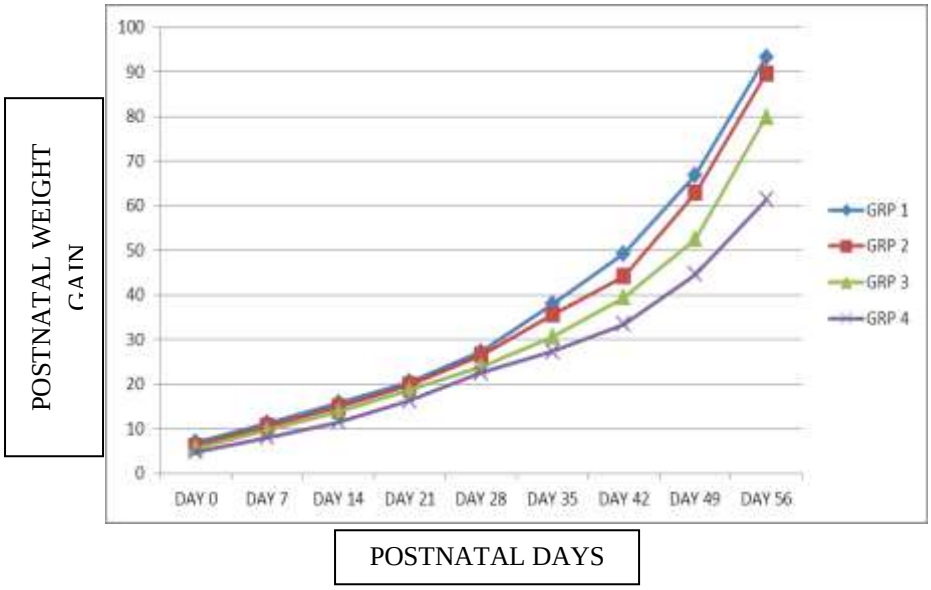

Figure 1: Effect of consumption of *A.indica* during pregnancy on postnatal weight gain of the offspring.

Aqueous leaf extract of *A. indica* showed significant decrease in postnatal weight gain from day 0 to postnatal day 56 in all the test groups when compared with the control group.

DISCUSSION

The present study has revealed that the maternal consumption of aqueous leaf extract of *A.indica* during pregnancy caused significant reduction in birth weight and postnatal weight gain in all the test groups when compared with the control group. This suggests that there was inadequate nutrient transfer across the placenta to the fetus (Boswell-Ruys *et al*, 2003). Epidemiological and experimental investigations had revealed that maternal nutrition during pregnancy has a significant effect on fetal development in-utero and later in life during development, and to maturity (Hoet and Hanson, 1999). Also, the significant reduction in birth weight observed may be associated with the earlier reported hypoglycemic activities of the leaf extract (Iyare and Obaji 2014, Ekaidem *et al*, 2003, Atangwho *et al*, 2010). This is

evident from the report of Pond (1996) that some anti-diabetic drugs induce intrauterine growth retardation (IUGR). Similar observation was recorded by Ogata *et al.*, (1987) and Lueder *et al.*, (1992) in which pregnant rats that suffered from insulin induced hypoglycemia delivered litters with significantly reduced weight compared to the control. The low mean birth weight revealed in this study could be attributed to the action of saponins, alkaloids and tannin, components of the extract causing impaired glucose supply to the fetuses and leading to intrauterine growth restriction (IUGR). Tannin has been reported to have anti-nutritional and toxic effects including reduced feed intake, growth rate, feed efficiency and net metabolizable energy (Acamovic *et al*, 2005, Jansman *et al*, 1993). It has also been reported to impair calcium absorption (Chang *et al*, 1994), which potentially could also affect bone metabolism. Since there was no birth anomalies observed, low birth weight observed here may be due to impaired glucose supply to the fetuses. This was also confirmed by Ravelli *et al*. (1998) who reported that reduced growth in-utero is linked to decrease glucose tolerance in adult life. This may support the use of this extract by pregnant women to make them feel "lighter" and also to prevent them from having "big babies". The observed effect is in agreement with work done by Ucheya and Ochei (2011) where they reported reduction in birth weight in neonates whose mothers were fed with leaf extract of *A.indica* during pregnancy.

Perinatal mortality is the death of a fetus in utero after the age of viability (still births) and deaths within the first seven days of life (early neonatal mortality) (WHO, 2006). It is an important vital statistics and an indicator of the level of foetal and maternal care. The increase in neonatal mortality observed could be as a result of the low birth weight observed in this study. Low birth weight has a major influence on neonatal morbidity, neurocognitive deficiencies, neurobehavioural effects and mortality (Han *et al*. 2000). Decrease in energy availability for placenta fetal transport during pregnancy will reduce energy available for fetuses. This has been well linked to the onset and development of intrauterine growth restriction (IUGR) and low birth weight. Low birth weight, preterm birth, and IUGR which are adverse birth outcomes represent the leading causes of neonatal death among children born without congenital anomalies (Bhutta *et al.*, 2005; Abu-Saad and Fraser, 2010).

CONCLUSION

Birth weight is closely associated with perinatal mortality and morbidity, and also with disease later in life (Wilcox *et al*, 1983, Godfrey *et al*, 2000). It may be a predictor for

survival as low birth weight infants have a greater risk of morbidity and mortality (Uthman, 2008; Dayina et al, 2010). Aqueous leaf extract of *A. indica* has the potential of reducing birth weight and postnatal weight gain. It also increases neonatal mortality especially at increased concentration. The result of this study therefore, discourages increased use of this leaf extract especially during pregnancy.

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