EFFECTS OF AQUEOUS EXTRACT OF AZADIRACHTA INDICA LEAF ON THE MATERNAL WEIGHT AND NEONATAL GROWTH IN ADULT WISTAR RATS

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ABSTRACT

The effect of Azadirachta indica leaf (neem leaf) on the maternal and Neonatal weight was conducted using 17 adult female wister rats with mean body weight ranging between 140 – 180g. They were allowed to acclimatize for 2 weeks, after which they were separated at random into four groups A, B and C of five rats each, group D was the control and was assigned only two rats, following which a male rat was introduced into each cage for mating. At the confirmation of pregnancy the female rats were administered graduating doses of aqueous extract of neem leaves. Group A received a dose of 350mg/kg/day of aqueous extract of neem, Group B received a dose of 700mg/kg/day of neem, Group C received a dose of 1100mg/kg/day of neem, while Group D served as the control group and received feed mash and saline water only. The results showed reduction in the weights of maternal animals administered 700mg/kg/day of aqueous extract of neem ($P < 0.01$) while maternal animals administered with 1100mg/kg/day of aqueous extract of neem leaf showed a reduction in mean body weight, and it was statistically significant (P , 0.001). The neonates of animals administered with 1100mg/kg/day of aqueous extract of neem leaf showed mean weight reductions as follows; mean body weight (14.10 ± 0.1); mean tail length (4.2 ± 0.2); mean crown-rump length (5.2 ± 0.3). These differences were all significant when compared to the control. Mean neonatal body weight ($P < 0.05$); mean neonatal tail length ($P < 0.01$); mean neonatal crown- rump length ($P < 0.01$). We suggest that administration of aqueous extract of neem at a high dose during pregnancy might be the aftermath of low birth weight and reduction in maternal weight after delivery. Conclusively, administration of aqueous extract of neem leaf at a high dose beyond 350mg/kg/day should be avoided especially in pregnant state inorder to prevent low birth weight and loss in maternal weight after delivery.

Keywords: Aqueous extract, Azadirachta indica, Birth weight, Pregnancy.

INTRODUCTION

The use of herbal medicine to cure ailments is as old as Homo sapiens. The common names of Neem are: Nim, Nimmi, vepa, Tamarka, Kohomba, Dogonyaro, Margosa and India Lilia. Botanical Names are: Azadirachta indica, and Melia azadirachta. The first indication that Neem was used as a medicinal plant was about 4500 years ago (Skeptics, 2004). Since then the populace at large has employed the use of unconventional medications without a foreknowledge of its adverse effects and safe dose (Foster, 1996). In ancient texts, neem was mentioned in almost 100 entries for treating a wide range of diseases and symptoms including malaria (Melanie, 1998). Malaria is a tropical disease that poses serious problems on human well-being especially in tropical countries where the environs provides conducive ground for the parasite to thrive well. It is a global problem because of migration and the mutant potential of the parasite in living organisms. Though undocumented there is a rampant usage of herbal medicine by the populace at large, amongst which is neem leaf extract, and this has prompted scientists to embarked on several researches as to how best they can be useful in curing ailments especially existing tropical diseases amongst which is malaria, and whose primary target organs are the liver, kidney and brain (Ucheya and Igweh, 2(010). Junquera et al. (1995) documented that there is a relation between total organ volume and organ weight expressed in a straight line on a logarithmic scale. Several drugs are in use in obstetrics and gynaecology, either in pregnant or non pregnant state, usually out of necessity, despite their reported toxicities and negative side effects (Simon, 1995). The case of thalidomide was a big disaster in the 1960s (Behrman et al, 1996).

However, in recent times, drug administration during pregnancy is done with utmost care. The clinical conditions necessitating the use of drugs during pregnancy include hypertention, thromboembolism, hyperthyroidism, epilepsy, diabetes mellitus, preterm labour, arthritis, pain fever and malaria, among others (Richard and Thomas, 1997).

Many workers had in the past also established the relationship between the use of herbal medicine, their curative potentials, beneficial importance and adverse effects. Neem seed and leaf extracts are effective against malarial parasites (Khalid, et al., 1989; Kahlid et al., 1986). Components of the alcoholic extracts of leaves and seeds are effective against both chloroquine-resistant and sensitive strains of malaria parasite (Badan et al., 1987). Recently, neem seed extract and its purified fractions have been shown to inhibit growth and development of asexual and sexual stages of drug sensitive and resistant strains of the human malaria parasite P. falciparum (Dhar et al., 1998). Udeinya et al, (2004) reported that anti malarial extract from neem leaves is antiretroviral. Ucheya and Biose (2010) in their research work on "The teratogenic

ffects of methanolic extract of Ricinus ommunis oil on the morphology of oetal wistar rats" reported that nethanolic Ricinus communis when onsumed during pregnancy might cause pontaneous abortion and reduced foetal arameters such as foetal weight; foetal rown length; and foetal tail length in vistar rats. Sinnial et al, (2003) also eported that neem leaf extracts and teas ppear to be very safe at recommended ntake levels with no significant reports of problems. Sinniah, et al (1995) stated that neem seed oil is more problematic and should be kept out of reach of children. Caius and Mhaskar, (1923) indicated that adults may sometimes have diarrhoea, nausea, or stomach upset when taking Neem oil. Caius and Mhaskar (1932) advised that neem should be avoided in pregnancy until its safety is established. Ucheya and Anibeze (2009) documented a report on the lethal dose studies (LD50 and LD100) of the aqueous extract of Azadirachta indica leaf, and possible morphological changes on the liver, kidney and brain (cerebellum). The above studies have shown the parasitic potentials of neem extracts and possible problems that might be associated with the use of neem extracts. This study is therefore aimed at determining the effects of aqueous extract of Azadirachta indica on the maternal and neonatal weight when used in pregnancy.

MATERIALS AND METHOD

Seventeen wistar rats with weight range (140g-170g) were procured from the animal house University of Benin, Ugbowo Campus and were randomly divided into four groups (A, B, C, and D) of five rats each, while group D was assigned two rats only and served as the control. They were kept in the Animal House of Anatomy Department, School of Basic Medical Sciences, for 2 weeks for acclimatization. They were housed in cages measuring 11 by 7cm and were allowed free access to food and water ad libitum.

Experimental Procedure

After two complete regular cycles, timed mating of the female animals was done on the night of the proestrous (N) phase of the cycle. In the morning following mating, vaginal smears were taken again. The presence of the Spermatozoa and squamus cells in the smear confirmed mating and fertilization of ovulated spermatozoa. The – positive morning was thus designated day 0 of pregnancy. Four groups of pregnant rats A, B, C and D were used. Graduated doses of the Experimental Herbal drugs were given to the experimental groups as follows:

Experimental group A – A safe dose of aqueous extract of Azadirachta indica 350mg/kg/body weight per day was given to each animal by oral route of administration and it was done by gavages. In experiment one, two and three, administration of Extracts started on the 1st day of gestation. This continued on a daily basis for 7days during gestation.

Experimental group B – was given a dose of 700mg/Kg/bodyweight per day of aqueous extract of Azadirachta indica. Administered was done by oral route to each animal by gavages. In experiment one, two and three, administration of Extracts started on the 1st day of gestation. This continued on a daily basis for 7days during gestation.

Experimental group C– This group was administered a high dose of aqueous extract of Azadirachta indica 1100mg/kg/body weight for the first seven days of pregnancy.

Experimental group D- This was the control group and received feed mash and water only.

Each rat was weighed at an interval of three days, before the experiment, and up to two weeks after the experiment. On the 13th day after parturition, the neonatal weights were recorded.

The mean weight of the animal before pregnancy was taken and designated as (a), then the weight on the 10th day of pregnancy at the unset of drug administration was taken and the mean weight for each group on the 13th day after parturition was taken and designated as (b), after which the mean weight at 13th day after parturition was subtracted from the mean weight before pregnancy for each animal group (a –b). The aqueous extract of A. indica leaf treated groups and the control group was then tested for a statistical significant difference using the T-test to compare two variance, (weight of aqueous extract treated animals versus weight of aqueous treated control animals). An Anova was used to test the variance in the three groups.

RESULTS

Effects of Aqueous Extract of A. indica on the Maternal Weight

The body weights of the maternal varied greatly during the experiments. Mean maternal body weight before the unset of the experiments, at the beginning of administration of drugs (10th day embryonic life) and at the conclusion of the experiments (15th day postnatal life), as shown in table 1. Animals that were administered with 350mg/kg/day of aqueous extract of A.indica had a 26.6% loss in weight compared to the control animals that had a 22.6% loss in weight, this loss was statistically not significant. The animals that were administered with 700mg/kg/day of aqueous extract of A.indica showed a lesser weight loss of 31.0% when compared to the control animal with a weight loss of 22.6%, this weight loss was statistically significant ($P < 0.01$). The animals that were administered with 1100mg/kg/day of aqueous extract of A.indica showed a more pronounced loss in weight (38.4%) when compared to the control, this was statistically significant ($P < 0.001$).

Effects of Aqueous Extract of A.indica on the neonatal weights, Tail Length and Crown-Rump Lenghts at 15th day of post nantal life.

There was no statistically significant difference found between the mean weight of the neonates of animals that were administered with 350mg/kg/day of aqueous extract of A.indica (17.0± 0.2) when compared to that of the control animals (17.1± 1.1). The mean weight of the animals treated with 750mg/kg/day of aqueous extract of A.indica leaf was (16.8± 0.5) and was not statistically significant when compared to that of the control animals (17.1± 1.1). The neonates of the animals administered with 1100mg/kg/day of aqueous extract of A.indica leaf showed a statistically significant difference between the mean tail lengths (4.2 ± 0.2cm) compared to the control (5.5 ± 0.2cm) There was no any statistically significant difference that was found between the crown-rump of the neonates of animals treated with 700mg/kg/day (6.4 ± 0.1cm) when compared to the control (7.2±0.2cm). However; there was a statistically significant difference between the mean Crown-rump length of neonates of animals that was administered 1100mg/kg/day of aqueous extract of A.indica leaf (5.2±0.3cm) when compared to the control animals (7.2±0.2cm). ▶

Figure 1: Effects of Aqueous Extract of Azadirachta indica on the Maternal Body Weight of Wistar Rats.

Animal Group	Drugs Administered	Weight Before Pregnancy (a)	Weight at 10th Day of Pregnancy	Weight at 13th Day after Parturition (b)	Mean Weight Difference (a – b)	% Weight Loss
A	350mg/day of Neem Extract	142.5±2.4	162.8±4.3	180.4±1.5	37.9±0.9	26.6
B	700mg/day of Neem Extract	180.0±3.1	191.0±3.8	235.8±2.1	*55.8±1.7	31.0
C	1100mg/day of Neem Extract	*153.3±2.5	173.6	212.2±3.6	**58.9±0.0	38.4
D	Feed Mash + H_2O (Control)	176.5±1.4	198.5±4.5	216.4±3.2	39.9±1.3	22.6

*Significantly different from value of control (*P <0.01; **P < 0.001; all weights in gram) Mean ± S.D

Figure 2: Effects of aqueous extract of Azadirachta indica on the neonatal weights, Tail Length, and Crown-Rump Lenghts at 15th day of post nantal life.

Animal Group	Extract Administered	Mean Neonatal Weight (g)	Mean Tail Length (cm)	Mean Crown-Rump Length (cm)
A	350mg/day of Neem Extract	17.0 ± 0.2	5.8±0.2	7.1 ± 0.2
B	700mg/day of Neem Extract	16.8 ± 0.5	5.3±0.1	6.4 ±0.1
C	1100mg/day of Neem Extract	**14.10±0.1	*4.2 ± 0.2	**5.2 ± 0.3
D	Feed Mash + H_2O (Control)	17.1±1.1	5.5±0.2	7.2 ± 0.2

*Significantly different from value of control (*P <0.05; **P < 0.01) Mean ± S.D

DISCUSSION

The current investigation reveals that the maternal rats showed drastic loss in weight, and this was more pronounced in rats receiving 1100mg/kg/day of aqueous extract of A.indica leaf (38.4%) than in those treated with 700mg/kg/day of aqueous extract of A.indica leaf (31.0%). The mean 38.4% loss in weight recorded for the animals that were treated with 1100mg/kg/day of aqueous extract of A.indica might have being due to the negative effect of the extract on the organ such as the liver, this is in conformity with the report that a high dose of aqueous extract of A.indica is toxic and has a degenerative effect on the histology of the liver (Ucheya and anibeze, 2009; Ucheya and Igweh, 2010). The apparent degeneration of the liver possibly could have induce the weight loss that was recorded in the maternal animals, this also is in agreement with the report that there is a relation between total organ weight and body weight expressed in a straight line on a logarithmic scale (Junqueira et al, 1995; Ucheya et al, 2005). Aqueous extract of A.indica leaf at a dose of 350mg/kg/day recorded a weight loss of 26.6%, on comparism with the weight loss recorded for the control animals (22.6%), it showed a mean weight difference (4%). However; this mean weight difference was not statistically significant when compared with that of the control animals. This finding is in total comformity with the report by Ucheya and Anibeze, (2009) in their research work on the lethal dose study on the aqueous extract of A.indica leaf in mice, they reported that aqueous extract of A.indica at a dose of 350mg/kg/day is safe on the histology of the kidney tissue of mice. It is also in conformity with the report that Herbal medications are safe when taken at a subtoxic dose and in their natural form (Malanie, 2004). It is evident from these observations that increasing dose of aqueous extract of A.indica leaf has from 700mg/kg/day has increasing effects on the weight of Adult female rats.

In addition, the neonatal body weights did not seem to be affected by 350mg/kg/day and 700mg/kg/day (Table 2) and no weight loss was recorded. Similarly, the tail length and crown-rump length did not differ significantly from control values. The neonates of the animals that were treated with 1100mg/kg/day of aqueous extract of A.indica leaf showed significant loss in body weight with reduction in tail length and crown- rump length, compared to the control values. These reductions could be due to the compromised nutritional status of the mother rats consequent to organ derangement that might have occur. This is in agreement with the report by Junqueira et al, (1995); Ucheya et al (2005), that there is a relation between total organ weight and body weight expressed in a straight line on a logarithmic scale.

Conclusively, the reduction in weight that was observed probably resulted from an overall derangement in the organ of the maternal and neonatal rats. Therefore

dministration of A.indica at a high dose eyond 350mg/kg/day of aqueous extract f A.indica should be avoided especially in regnant state inorder to prevent low irth weight.

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