ORIGINAL ARTICLE

Mental and Growth Retardation following Prenatal Exposure to Oral Bitter Leaf (*Vernonia amygdalina*) Extract in Developing Wistar Rats

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ABSTRACT

Objective: The teratogenic influence of maternal exposure to bitter leaf on the morphology of foetal nervous system was studied.
Methodology: In this study, we used twenty five pristine female Wistar rats of an average weight of 215g. The rats were distributed into 5 groups with each having 5 rats. A separate cage was used to keep each group. Overnight, the females were kept with sexually active male of the same species. The following morning, Vagina smears was done on the female to confirm coitus, the presence of tailed structures in the smears was taken as sperm positive day and was taken as day zero of pregnancy. 400mg/kg of bitter leaf were administered orally between gestational days 1-7 (group B), 8-14 (group C), 15-21 (group D) and 1-21 (group E). The control (group A) was administered 2.0ml/kg/bw of normal saline throughout pregnancy. After parturition, the brain and body weights of the litters were recorded on postnatal days 1, 7, 14, 21, 28 and 35.
Results: Results show that the litters of the group C and E demonstrated significant ($p < 0.05$) reduction in all parameters measured when compared to the control and the group B and D. The foetal weight and brain weight decreased significantly ($p <0.05$) when compared with the corresponding values in the control group. The body to brain ratios of group C and E also show variation when compared to the control.
Conclusion: Our results suggest that administration of bitter leaf during the second week of pregnancy may retard growth of the body and brain in Wistar rats.

Key words: Cerebral cortex, brain weight, body weight, wistar rats.

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INTRODUCTION

Teratology is the branch of science that studies the causes, mechanisms, and pattern of abnormal development[1]. The abnormal developments that are associated with teratology maybe with or without morphological defects but always have slow or late mental development with growth retardation[2]. When an organism is expose to teratogenic agents, various deformities can occur subject to the stage of development at the time the organism was attacked[3].

High dose or indiscriminate intake of medicinal plants have been associated with some negative effects on the body especially during pregnancy[4]. It has been shown to have teratogenic effects on the foetuses, which were exposed during various gestational stages[4]. Bitter leaf (*Vernonia amygdalina*) is a common local plant in Africa especially Nigeria because of its therapeutic and nutritional properties and is reported as the most used medicinal plant from the genus *Vernonia*[5]. It is used for different functions in Nigeria. The water extract of the leaves is used as tonic for managing blood sugar and for rejuvenating vital organs like the kidney[6]. Ademola and Eloff[7] reported that bitter leaf also possess anthelminthic anti-parasitic properties. Akinola et al.,[8] reported the anti-diabetic and non-cytotoxic nature of bitter leaf on the liver. Bitter leaf have also been shown to provide anti-oxidant benefits[9].

Bitter leaves are being used to treat various diseases at different stages of pregnancy. It has been found that there is a powerful uterotonic component in bitter leaf such that a non-alcoholic extracts of the plant (100 mg/mL) can induce uterine contraction amplitudes in guinea pig dams similar to what is seen in ergometrine[10].

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In Guinea-Bissau and Nigeria, the infusion of leaves of *Vernonia amygdalina* is used as an abortifacient in women[11]. Desta[12] also reported that ethanolic extract of bitter leaf possessed uteronic (2%) and anti-implantation (1.54g/kg) consequence.

High dose or indiscriminate intake of medicinal plants has also been associated with some negative effects on the body especially during pregnancy. It has been shown to have teratogenic effects on the offspring of mothers who took them at various gestational periods[4]. Currently, plants are being screened for their efficacy, safety and dosage in pregnancy. Several plants like corn lily and marijuana are known to be teratogenic in nature especially during the first trimester of pregnancy[13,14]. The present work investigated if the use of bitter leaf is deleterious to the developing embryo.

METHODOLOGY

Animal handling:

Twenty five pristine female Wistar rats of an average weight of 215g bred in the animal holding of the Department were used in this study. The rats were distributed into 5 groups with each having 5 rats. A separate cage was used to keep each group. Rat feed from Benue rat feed (Ewu, Nigeria) and tap water were served the rats ad libitum throughout the study period. On the pro-oestrous day when the females were receptive to males, they were kept overnight with sexually active male rats at ratio 2 females to 1 male. The following morning, vaginal smears were done on the females to confirm coitus and, the presence of tailed structures in the smears was taken as sperm positive day. The sperm positive day was taken as day zero of pregnancy. Throughout this study period, rats were maintained with the standard of Institutional Animal Care and Use Committee (IACUC). Ethical clearance was obtained from the ethical committee of the faculty of basic medical science of the university.

All institutional and national guidelines for the care and use of laboratory animals were followed. All research work has been performed in accordance with the standard code of ethics for animal experiments as described at http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm

Extract preparation:

Vernonia amygdalina fresh leaves were procured from a local market in Ilorin, Kwara State before authenticated at the Plant Biology Department of the University of Ilorin, Ilorin, Nigeria. 250g of the leaves were weighed out before they were air dried, weighed, pound, reweigh and sieved. 100g part of this powdered leaf was weighed out and softened in 1L of distilled water. The concoction was stirred and placed on the laboratory bench for 24 hours before sieving through the Whatmann's no 1 filter paper (Maidstone, UK). The deposit was evaporated to dryness at 40°C in a Rotary evaporator. 10g of a paste-like dark coloured substance was obtained[15].

Animal grouping:

Oral doses of 400mg/kg of bitter leaf extract were administered between gestational days 1-7 (group B), 8-14 (group C), 15-21 (group D) and 1-21 (group E). The control (group A) received 2.0ml/kg/bw of normal saline throughout pregnancy.

Animal sacrifice:

After parturition, the litters were euthanized on postnatal day 1, 7, 14, 21, 28 and 35 after parturition. The weights of the litters were taken before sacrifice while the brain weight was measured after each sacrifice with a sensitive weighing balance.

Statistical analysis:

Outcomes of the morphological observations were conveyed as Mean ± SEM. ANOVA was used to perform the statistical analysis while $p < 0.05$ was used as statistically significant after which Duncan post-hoc test was done.

RESULTS

To check the effect of the extract of *Vernonia amygdalina* on the growth of the body and brain of developing rats, we measured the body weight, brain weight and brain to body ratio of the rats. The following effects were observed.

Table 1: showing number of litters obtained from each group

Groups	Number of mother rats	Total no of litters
A	5	47
B	5	52
C	5	45
D	5	43
E	5	44

Effects on body weight:

The body weight was measured for 7 weeks after birth. It was discovered that the litters of the mothers treated with bitter leaf in the group C and E increased significantly from postnatal day 14 when compared to

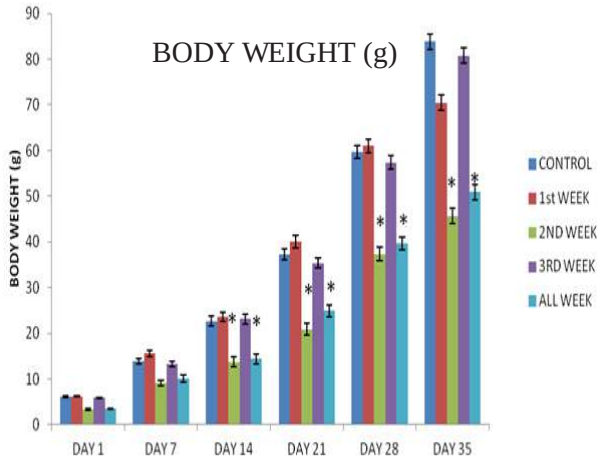

Figure 1: Mean body weight of litters (in grams). Values are Mean ± SEM. * represent P<0.05

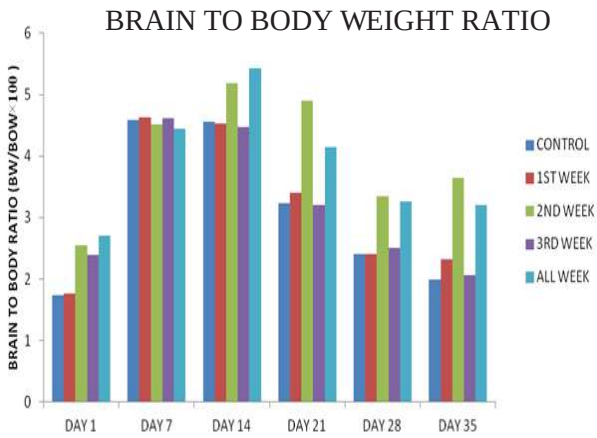

Figure 3: Mean brain to body weight of litters.

the control ($p < 0.05$). In contrast, the levels of those treated in group B and D were similar to the control group. Figure 1 shows body weight in the various bitter leaf treatment groups compared to control at each postnatal day of sacrifice.

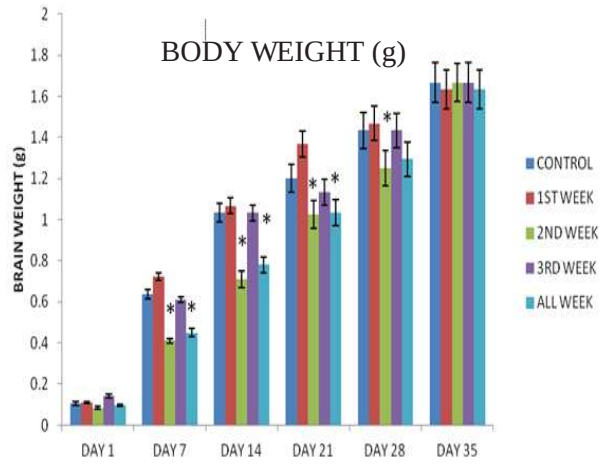

Figure 2: Mean brain weight (in grams) of litters. Values are Mean ± SEM. * represent P<0.05

Effect on brain weight:

The litters of the mothers treated with bitter leaf in the group C and E increased significantly from day 7 postnatally in comparison to the control ($p < 0.05$), from day 28 postnatally, the litters in group E was similar to the control while on postnatal 35, the group C was similar to the control. In contrast, the levels of those treated in group B and D were similar to the control group. Figure 2 shows brain weight in the various bitter leaf treatment groups compared to control at each postnatal day of sacrifice.

Effect on brain to body weight ratio:

There was fluctuation in the group C and E when compared to the control while the group B and D appears similar to the control. Figure 3 shows the brain weight to body weight ratio in the various bitter leaf treatment groups compared to control at each postnatal day of sacrifice.

DISCUSSION

The use of medicinal herbs in traditional system of medicine is a common practice in many cultures around the world, especially in African society[16]. This practice has gained widespread acceptance in developing as well as developed nations. Researchers are also beginning to appreciate the role of medicinal plants in health care delivery. This is as a result of the effectiveness, low cost and the availability of these herbal medicines[17]. It is noteworthy that some orthodox medicines in use today were developed from the biochemical templates obtained from medicinal plants[16].

Medicinal plants contain compounds that have prominent effects on the animal system and some possess important therapeutic properties which can and have been used in the treatment and cure of human diseases[18]. The use of herbs in their processed and unprocessed form is far becoming popular in our society. Unfortunately, misconceptions regarding safety and efficacy of these drugs are common, also doses used may be much higher than those considered safe[18]. Plants have positive and negative effect on animals due to substances present in them. However, some plants when taken in excess will have adverse effect and might lead to damage or loss of function of a particular organ in the body[4].

Noback and Demarest[19] discovered that as high as 50% cases of congenital deformities and atypical development include the nervous system. In experimental animals, deformities of the nervous system usually occur when the teratogen is given shortly earlier or during the closing of the neural groove[20,21].

The body weights of the litters exposed to bitter leaf on E8-14 and E1-21 reduced significantly ($p < 0.05$) in comparison to the control group. This shows that the extract had effect on the weight of the litters. The administration of bitter leaf during pregnancy might result into low body weight associated with teratogens.

The brain weight of the litters were also checked, the brain of the litters exposed on E8-14 and E1-21 were found to significantly decreased ($p < 0.05$) from the control group, this may have occurred because the extract was given at main stages of brain development which have been reported to start on embryonic day 11 in rats[22,23]. The low body and brain weight seen in the litters exposed to bitter leaf during the E8-14 and E1-21 is in consonance with previous studies that teratogenic insults on the emerging brain during neurogenic period causes anomalies[24,25].

The brain to body weight ratio shows that the brain of the litters of the second and all week was not developing relatively to the body. This implies that there is disequilibrium in the growth of the litter's brain and the body weight. The ratio has been reported to be a rough estimate of an animal intelligenc[26].

The most vulnerable period for malformation during embryogenesis is usually during organogenesis period[1]. Because of the very unstable and flexible nature of the cells at this phase of development, they are usually vulnerable to injurious effects, which extremely obstruct, delays or absolutely halt their developmental processes. It has been discovered that the critical period of foetal development in rodents is established to be between days 6 to12 of their gestation period[27]. This is the second week where a lot of malformations were seen.

The mechanism by which bitter leaf act as a teratogen is unclear but phytochemistry shows that it contain some constituents like alkaloids and flavonoids[10] which have been reported to cross the placental and affect developing embryo, further studies need be done to know the exact constituent that affects the developing embryo. Based on the observations from this study, it can be concluded that the exposure to 400 mg/kg of oral bitter leaf during the second week of pregnancy can cause low body and brain weight.

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