



Effects of Aqueous Leaf Extract of *Annona muricata* on Pregnancy and Pregnancy Outcome

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EO, IE and AUB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EO and IE managed the analyses of the study. Authors OP and UP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Indiscriminate and increasing consumption of herbal medicinal products by pregnant women presents major concern due to paucity information on safety. *Annona muricata* also known as soursop is commonly consumed in Nigeria including by pregnant women, but information remains poor on effects on pregnancy outcomes.

Aims and Objectives: This study was carried out to determine the effect of the aqueous extract of *Annona muricata* leaves on maternal weight gain during pregnancy, placental weight, litter size, body weight at birth and placental/body weight ratio in female Wistar rats.

Materials and Methods: Twenty-four (24) female Wistar rats were used for this study. On confirmation of pregnancy by presence of spermatozoa in vaginal smears and vaginal plugs in cages, the rats were then divided into four groups of six rats each receiving different concentration of the extract as follows; 15 mg, 30 mg, and 45 mg/kg of body weight. The extract was administered

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orally and daily throughout the gestational period of the Wistar rats. The maternal body weights were measured daily; before extract administration, during extract administration and prior to sacrifice. On day 18 of pregnancy, 4 rats from each group were sacrificed; placentae harvested and weighed using analytical weighing balance. The foetal number in utero were also determined and recorded. On the day of delivery for the remaining 2 rats, the gestational length, litter size and birth weight were recorded and litter length measured to the nearest (cm) using a measuring tape. Morphological appearance of the offspring was also assessed.

Statistics: Data obtained as mean \pm standard deviation were analysed using one way analysis of variance (ANOVA) followed by a post hoc student's Neuman-Keul's test. Level of significance between groups was taken as $p < 0.05$.

Results: There was a significant reduction in maternal weight during pregnancy, litter birth weight, litter length, placental weight and placental/birth weight ratio of the extract-treated pregnant group ($p < 0.05$) compared to control group.

Conclusion: Consumption of *Annona muricata* by pregnant women can significantly reduce the utero-placental transfer of nutrients and as a consequence impair fetal growth.

Keywords: Pregnancy; herbal; *Annona muricata*; Nigeria.

1. INTRODUCTION

Herbal remedies from parts of plants or other plant materials contain active ingredients perceived to have therapeutic benefits with their use dating back to prehistoric days. The use of Herbal medicines, including herbs, herbal preparations, and finished herbal products appear to be on the increase worldwide especially among pregnant women [1,2]. This rising utilization by pregnant women has also been reported in Nigeria and is believed to be due its relatively low cost, increasing media publicity, traditional believe of herbs being natural, so safer to use during pregnancy than conventional medicines, cultural belief on multiple therapeutic abilities and easy accessibility [3,4]. The use of herbal drugs in pregnancy presents special concern because paucity of information on the risks/benefits due to absence of pre-registration clinical trials and post approval surveillance [4]. It is clear thus that herbal products in pregnancy may pose dangers to the fetus and parturient [4]. In Nigeria, herbal products are not registered as drugs and legislation for distribution and purchase of herbal medicines is not as stringent as it is for conventional medicines.

Annona muricata also known as *soursop*, *graviola* and *guanabana* is a member of the Annonaceae family [5,6]. It is an evergreen fruit tree mostly distributed in tropical and subtropical regions of the world. The seeds, fruit, and leaves have very long history of traditional use for many different ailments [7] Documented uses of parts of the plants include utero-constrictive [8]; anti-malarial, anti-mutagenic (cellular protector),

emetic (induce vomiting), anti-convulsant [9,10]; anti-hypertensive via vasodilation or and cardio depressing activities [11]; anti-hyperglycemic [12]; and anti-pyretic and anti-viral activity [13]. The leaves of *A. muricata* from Nigeria are reported to be a reservoir for free radical scavenging molecules such as vitamins, alkaloids, tannins, terpenoids, phenolic acids, flavonoids and other metabolites, which are basically rich in antioxidant activities [14]. Studies from Nigeria also exists on beneficial and therapeutic effects of the leaves of *A. muricata* as erythroietic [15], anti-diabetic [16] and anti-fungal [17] but limited information is currently available on the effects during pregnancy and in pregnancy outcome. This study was carried out to determine the effect of the aqueous extract of *Annona muricata* leaves on maternal weight gain during pregnancy, placental weight, litter size, body weight at birth and placental/body weight ratio in female Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Annona muricata* were harvested (collected) from the Enugu State, Nigeria. They were subsequently identified and authenticated by a Botanist of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

2.2 Preparation of Extracts

The extraction was according to the method of Cowan 1999 [17]. *A. muricata* leaves were air

dried at room temperature for four weeks. The dried leaves weighed, milled into fine powder and stored in a dry place. 1.19 kg of the dried powdered leaves was soaked into 1.5 L of distilled water at 60°C to prevent fungal growth and cooled at room temperature. After 24 hours, the mixture was filtered using whatman 1 filter paper and the filtrate was concentrated to a paste using rotatory evaporator at 50°C for about 15 hours. The yield was 32.96% (weight of plant material was 1190.59 g and total weight of extract was 392.37 g). It was then stored in an air-tight container in the fridge and was reconstituted for each treatment group respectively.

2.3 Care and Management of Animals

Twenty-four adult nulliparous female Wistar rats weighing between 200-300 g were used for this study. The rats were obtained from animal house of University of Nigeria, Nsukka. They were weighed, randomly assigned into metallic well ventilated cages and kept in a room with a 12 hours light/dark cycle. They were allowed free access to food (super starter feed) and water and were allowed for 1 week to acclimatize before commencement of the study. The care for the rats were according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

2.4 Experimental Grouping and Treatment

The rats were randomly selected and divided into four groups of 6 rats each. To each group, three (3) matured male rats of proven fertility were introduced allowed to stay for a period of 4 days in rats for mating (ratio of 2 female rats to 1 male). Vaginal smear was examined under a microscope every morning, a successful mating was ascertained by spermatozoa seen in the vaginal smear of the female rats and this was regarded as day 1 of pregnancy [10].

The method of administration of extract adopted was oro-gastric intubation using a cannula. Each animal in the treatment group was administered a volume of the extract in accordance with the dosage for its group, for a period of the pregnancy duration. The dosage of the extracts was used to group the animals.

Extracts were administered as follows;

- Group A: Pregnant control (feed +water only)
- Group B: 15 mg/kg of the body weight
- Group C: 30 mg/kg of the body weight
- Group D: 45 mg/kg of the body weight

2.5 Measurement of Parameters

The maternal body weights were daily before, during and after pregnancy using a digital electronic compact balance and recorded to the nearest gram.

On day 18 of pregnancy, 3 rats from each group were sacrificed; placentae harvested and weighed using analytical weighing balance.

The foetal number in utero were determined and recorded. On the day of delivery for the remaining 3 rats, the gestational length, litter size and birth weight were recorded and litter length measured to the nearest (cm) using a measuring tape. Morphological appearance of the offspring was assessed.

2.6 Statistical Analysis

The results were presented in tables as mean \pm standard deviation. Data obtained from study were analysed using SPSS version 20.0. Data analysis were done by one way analysis of variance (ANOVA) followed by a post hoc student's Neuman-Keul's test. Level of significance between groups was taken as $p < 0.05$.

3. RESULTS

3.1 Effects of Oral Administration of Aqueous Leaf Extract of *A. muricata* on Maternal Weight

There was a significant reduction in the maternal weight in the extract treated groups compared with the control ($p < 0.05$) (Table 1).

3.2 Effects of Oral Administration of Aqueous Leaf Extract of *A. muricata* on Placental Weight

The extract significantly reduced the placental weight when compared with control group (Table 2).

Table 1. Effect of oral administration of aqueous leaf extract of *A. muricata* on maternal weight during pregnancy

Groups	Maternal weight (g) (First trimester)	Maternal weight (g) (Second trimester)	Maternal weight (g) (Third trimester)
Control	315.35± 4.95	327.85± 4.64	337.35±3.27
15 mg/kg body weight	302.41± 5.61*	330.20± 5.38	318.56± 2.39*
30 mg/kg body weight	307.08± 5.33	312.85± 4.75*	330.35±4.75
45 mg/kg body weight	294.91± 5.46*	315.02± 3.22*	332.83± 5.75

Values are expressed as mean ±SD, *P< 0.05 compared with control

Table 2. Effects of oral administration of aqueous leafextract of *A.muricata* on Placental weight

Groups	Placental weight
Control	0.57±0.01
15 mg/kg body weight	0.50±0.01*
30 mg/kg body weight	0.46±0.01*
45 mg/kg body weight	0.38±0.01*

Values are expressed as mean ±SD
*P< 0.05 compared with control

3.3 Effects of Oral Administration of Aqueous Extract of *A. muricata* on Litter Size at Birth, Litter Weight and Litter Length

The extract did not have any significant effect on litter size of the treatment group when compared with control (Table 3). However, extract significantly reduced the litter birth weight across all the extract treated groups when compared with the control group. The extract also reduced the litter length significantly when compared to control.

3.4 Effects of Oral Administration of Aqueous Leaf Extract of *A. muricata*. Placental/Birth Weight Ratio

The extract caused a significant reduction in the placental/birth weight ratio of the treatment groups when compared with the control group (Table 4).

4. DISCUSSION

The use of herbal medicines in pregnancy is a matter of concern constitutes a major challenge for health care providers as most of them are not made aware of their use [18,19] Despite the lack of sufficient evidence on the safety profile of herbal products in pregnancy, it is increasingly used by expectant mothers. Indeed, pregnancy-related health problems, including nausea,

vomiting, constipation, and heartburn may result in pregnant women self-medicating using over-the-counter (OTC) medications, seeking prescribed medications, or using herbs [19].

Placental blood flow can affect pregnancy outcomes [20,21]. Present studies documents that aqueous extract of *Annona muricata* caused significant weight loses in different doses at the three (3) trimesters (Table 1). The mechanism of the weight reducing effect of the aqueous extract of this plant leaves is not clear but may be due to the presence of alkaloids which has been linked in previous studies [22]. The extract was also noted to have caused a significant reduction on placental weight of the treated rats across all groups when compared with the control (Table 2). It is possible that treatment with *Annona muricata* could cause a reduction in placental blood flow and hence reduction in adequate placental maturation and growth. This mechanism of reduction in placental weight might not be unconnected with alkaloid constituent of the extract which has been reported to produce this effect [22]. This reduction in placental weight could also be linked to the phenolic content of the extract which has been reported to have this effect [22].

Other significant findings from this study are the effect of extract on pregnancy outcomes as seen in Table 3. Though the extract had no significant effect in litter size, it demonstrated significant reduction in both Litter Weight and Length of offsprings at the different doses. This significant reduction in the biometrics could be attributed to the effect of the extract on the maternal decidua; placental bed as discussed above and subsequently reducing flow and transfer of nutrients to the foetus. The placenta is actively involved in the integration of nutritional signals from the maternal to foetal axis [23]. The ability of the foetus to thrive and grow in utero is largely determined by the availability of nutrients to the foetus via the placenta. This weight of placenta is also an important determinant of both birth weight and foetal growth [24].

Table 3. Effect of Oral administration of aqueous extract of *A. muricata* on litter size at birth, litter weight and litter length

Group	Litter size	Litter weight (g)	Litter length (cm)
Control	5.33± 0.33	4.60± 0.07	4.84± 0.02
15 mg/kg body weight	4.67± 0.33	4.38± 0.04*	4.88± 0.01*
30 mg/kg body weight	5.0± 0.58	4.37± 0.01*	4.85± 0.01*
45 mg/kg body weight	5.33± 0.67	4.19± 0.01*	4.75± 0.01*

Values are expressed as mean± SD

*P< 0.05 compared with control

Table 4. Effects of oral administration of aqueous leaf extract of *A. muricata* on placental/birth weight ratio

Groups	Placental/birth weight ratio
Control	0.125±0.002
15 mg/kg body weight	0.114±0.002*
30 mg/kg body weight	0.104±0.001*
45 mg/kg body weight	0.090±0.001*

Values are expressed as mean ±SD

*P< 0.05 compared with control

The relationship between the placenta and the foetal weight has been found to have a positive correlation with the ratio between placenta and newborn weight been reported as 1:6 [19]. It has also been found that maternal weight gain during pregnancy has a positive influence on foetal growth and pregnancy outcome [22].

In this study, there was a significant reduction in maternal weight gain, placental weight and litter weight of pregnant rats treated with the aqueous extract of *Annona muricata*. Studies show that crystalline alkaloid obtained from ephedras causes vasoconstriction in the utero-placental vascular bed, which probably could reduce placental blood flow; nutrient transfer is subsequently reduced and as a consequence impair foetal growth (litter and placental weight) as observed by previous studies [23]. Infant underweight is due to reduction in the ability of the placenta to transfer nutrients adequately to foetus [22,24]. Alkaloids (ergot) have been found to reduce maternal weight gain during pregnancy [23]. These may be the rationale behind this alkaloid rich extract *Annona muricata* exhibiting significant reduction in maternal weight gain, placental weight and litter weight.

Furthermore, the reduction in placental weight in the extract treated group could also be attributed to the phenolic content of the extract. Phenol which is an active constituent of the extract, has been reported to reduce placental weight by significantly reducing levels of placental lactogen, prolactin-like protein A and C, and decidual

prolactin related protein [16]. This may be the rationale behind the extract causing significant reduction in placental weight.

5. CONCLUSION

In conclusion, the aqueous leaf extract of *Annona muricata* was found to reduce the maternal weight, placental weight, placental/birth weight ratio and foetal biometric parameters (birth weight and length), possibly through some of the phytochemical constituents. These reported effects highly suggest that the use of this extract during pregnancy is unsafe and should be largely discouraged.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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