

Original Research Article

Effects of aqueous leaf extract of *Annona Muricata* on pregnancy and pregnancy outcome

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 widely consumed by pregnant women in Nigeria, 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: Pregnancy was induced in Twenty-four (24) female wistar rats which were subsequently divided into four groups of six rats each receiving different concentration of the extract as follows; control, 15mg, 30mg, and 45mg/kg of body weight. The extract was administered orally and daily throughout gestation. The maternal body weights were measured daily before, during and after pregnancy. 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 NeumanKeul's test. Level of significance between groups was taken as $p \leq 0.05$.

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.

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.

Annonamuricata 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-constrictive⁸; anti-malarial, anti-mutagenic (cellular protector), emetic (induce vomiting), anti-convulsant^{9,10}; anti-hypertensive via vasodilation or and cardio depressing activities¹¹; anti-hyperglyceamic¹²; and anti-pyretic and anti-viral activity¹³. 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 erythroietic¹⁵, anti-diabetic¹⁶; 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.

MATERIALS AND METHODS

Plant Material

The leaves of *Annonamuricata* were harvested (collected) from the Enugu State, Nigeria. They were subsequently identified and authenticated by a Botanist of the Department of Plant

65 Science and Biotechnology, University of Nigeria, Nsukka. Voucher number UNH Number 1 was
66 assigned to it.

67 Preparation of Extracts

68 The extraction was according to the method of Cowan 1999.¹⁷ *A. muricata* leaves were air dried
69 at room temperature for four weeks. The dried leaves weighed, milled into fine powder and
70 stored in a dry place. 1.19kg of the dried powdered leaves was soaked into 1.5L of distilled
71 water at 60 °C to prevent fungal growth and cooled at room temperature. After 24 hours, the
72 mixture was filtered using whatman 1 filter paper and the filtrate was concentrated to a paste
73 using rotatory evaporator at 50°C for about 15 hours. The yield was 32.96% (weight of plant
74 material was 1190.59g and total weight of extract was 392.37g). It was then stored in an air-
75 tight container in the fridge and was reconstituted for each treatment group respectively

76 Care and Management of Animals

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

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86 Experimental Grouping and Treatment.

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

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

- 97 Extracts were administered as follows;
- 98 Group A: Pregnant control (feed +water only)
- 99 Group B: 15mg/kg of the body weight
- 100 Group C: 30mg/kg of the body weight
- 101 Group D: 45mg/kg of the body weight

102 Measurement of Parameters

- 103 The maternal body weights were daily before, during and after pregnancy using a digital
- 104 electronic compact balance and recorded to the nearest gram.
- 105 On day 18 of pregnancy, 4 rats from each group were sacrificed; placentae harvested and
- 106 weighed using analytical weighing balance.
- 107 The foetal number in utero were determined and recorded. On the day of delivery for the
- 108 remaining 3 rats, the gestational length, litter size and birth weight were recorded and litter
- 109 length measured to the nearest (cm) using a measuring tape. Morphological appearance of the
- 110 offspring was assessed.

111 Statistical Analysis

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

116 RESULTS

117 EFFECTS OF ORAL ADMINISTRATION OF AQUEOUS LEAF EXTRACT OF *A.MURICATA* ON 118 MATERNAL WEIGHT

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

121 EFFECTS OF ORAL ADMINISTRATION OF AQUEOUS LEAF EXTRACT OF *A.muricata* ON PLACENTAL 122 WEIGHT.

- 123 The extract significantly reduced the placental weight when compared with control group (table
- 124 2).

125 EFFECTS OF ORAL ADMINISTRATION OF AQUEOUSEXTRACT OF *A.muricata* on LITTER SIZE AT
126 BIRTH, LITTER WEIGHT AND LITTER LENGTH.

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

131 EFFECTS OF ORAL ADMINISTRATION OF AQUEOUS LEAF EXTRACT OF *A.MURICATA*.
132 PLACENTAL/BIRTH WEIGHT RATIO

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

135 DISCUSSION

136 The use of herbal medicines in pregnancy is a matter of concern constitutes a major challenge
137 for health care providers as most of them are not made aware of their use¹⁸ Despite the lack of
138 sufficient evidence on the safety profile of herbal products in pregnancy, it is increasingly used
139 by expectant mothers. Indeed, pregnancy- related health problems, including nausea, vomiting,
140 onstipation, and heartburn may result in pregnant women self-medicating using over-the-
141 counter (OTC) medications, seeking prescribed medications, or using herbs.¹⁹

142 Present studies documents that aqueous extract of *Annona muricata* caused significant weight
143 loses in different doses at the three (3) trimesters (Table 1). The mechanism of the weight
144 reducing effect of the aqueous extract of this plant leaves is not clear but may be due to the
145 presence of alkaloids which as been linked in previous studies²⁰. However though many alkalids
146 containing known. It is however important to note reviews show that though some studies
147 provide encouraging data but no evidence beyond a reasonable doubt that any specific dietary
148 supplement is effective for reducing body weight

149 The extract was also noted to have caused a significant loss

150 placental weight of the treated rats across all groups when compared with the control (table 2).

151 It is possible that treatment with *Annona muricata* could cause a reduction in placental blood
152 flow, and hence reduction in adequate placental maturation and growth. This mechanism of
153 reduction in placental weight might not be unconnected with alkaloid constituent of the extract
154 which has been reported to produce this effect.²¹ The reduction in placental weight could also
155 be linked to the phenolic content of the extract which has been reported to have this effect.²²

156 Other significant findings from this study are the Effect of Extract on pregnancy outcomes as
157 seen in table 3. Though the ectract 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

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.²⁰

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 *Annonamuricata*. Studies show that crystalline alkaloid obtained from *Mephedras* 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.²¹ Infant underweight is due to reduction in the ability of the placenta to transfer nutrients adequately to foetus.²² Alkaloids (ergot) have been found to reduce maternal weight gain during pregnancy.²³

This may be the rationale behind this alkaloid rich extract *Annonamuricata* 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 and this may be the rationale behind the extract causing significant reduction in placental weight.

CONCLUSION

In conclusion, the aqueous leaf extract of *Annonamuricata* 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. Hence, by these effects, the use of this extract during pregnancy is unsafe and should be largely discouraged.

REFERENCES

1. World Health Organization. Traditional medicine. Geneva. 2008. Available at <http://www.who.int/medicines/areas/traditional/en/>. Accessed August 7, 2015.
2. John LL* and Shantakumari N Herbal Medicines Use During Pregnancy: A Review from the Middle East. *Oman Medical Journal* 2015 Jul; 30(4): 229–236
3. Fakeye TO, Adisa R and Musa IE Attitude and use of herbal medicines among pregnant women in Nigeria *BMC Complementary and Alternative Medicine* 2009 9:53
4. Aniebue PN, Aguwa CC, Aniebue UU The Use Of Herbal Drugs In Pregnancy By Women Receiving Antenatal Care In Tertiary Hospitals In Enugu, Nigeria. *Journal of Experimental Research* December 2014, 2(2): 73-80
5. Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM and Kadir HA *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities *Int J Mol Sci.* 2015; 16(7): 15625–1565
6. Adeyemi DA, Komolafe OA, Adewole OS, Obuotor EM and Adenowo TK. Anti Hyperglycemic Activities of *Annona Muricata* (Linn). *Afr J Tradit Complement Altern Med.* 2009; 6(1): 62–69.
7. Feng PC. Pharmacological screening of some West Indian medicinal plants. *J Pharm Pharmacol.* 1962;14:556–56
8. Marian Lozoya and Xavier Lozoya. A Uterotonic Substance from *Annona Cherimola* Seeds *Am. J. Chin. Med.* 1980; 08, 268 ().
9. Somsak V, Polwiang N, Chachiyo S. *Journal of Pathogens.* 2016 Mar 22; 2016: 3264070
10. N'gouemo P. Effects of ethanol extract of *Annona muricata* on pentylenetetrazol-induced convulsive seizures in mice. *Phytother Res.* 1997;11(3):243–245
11. Feng PC. Pharmacological screening of some West Indian medicinal plants. *J Pharm Pharmacol.* 1962;14:556–561.
12. Adeyemi D), Komolafe OA, Adewole OS,¹ Obuotor EM and Adenowo TK. Anti Hyperglycemic Activities of *Annona Muricata* (Linn) *Afr J Tradit Complement Altern Med.* 2009; 6(1): 62–69.
13. Padma P, Pramod NP, Thyagarajan SP, Khosa RL. Effect of the extract of *Annona muricata* and *Petunia nyctaginiflora* on Herpes simplex virus. *J Ethnopharmacol.* 1998 May; 61(1):81-
14. Usunobun U, Okolie N P., Anyanwu OG, Adegbegi AJ and Egharevba M E. Phytochemical screening and proximate composition of *Annona Muricata* Leaves. *European Journal of Botany Plant Science and Phytology* 2015; 2 (1): 18-28

15. Agbai EO, Emmanuel PN and Nwafor A. The Beneficial Effects of Methanol Extract of Annona Muricata Seeds on Acute Hemolytic Anemia in Albino Wistar Rats. JMSCR 2014; 2
16. Agbai EO. Nwanegwo CO. Effect of methanolic extract of annona muricata seeds on liver function enzymes in alloxan induced diabetic male mice. J Agric Sci Policy Res. 2013;3(2):46-59
17. Cowan MM. Plant Products as Antimicrobial Agents. Clin. Microbiol. Rev.1999, 12(4):564
18. Adams J. Growing popularity of complementary and alternative medicine during pregnancy and implications for healthcare providers. Expert Rev Obstet Gynecol. 2011;6(4):365-366
19. Wells BG. Gynecologic and obstetric disorders. In: Wells BG, Dipiro JT., Swinghammer, LT, Dipiro CV. Pharmacotherapy handbook. 7th Edition. The McGraw-Hill Companies. United States of America, 2009
20. Adejuwon AA., Peter AC., Weight losing, antihyperlipidemic and cardioprotective effects of alkaloid fraction of Hunteria umbellata seed extract on normal and triton-induced hyperlipidemic rats. Asian Pacific Journal of Tropical Biomedicine.2015;5:387-94
21. Reynolds LP¹, Redmer DA. Utero-placental vascular development and placental function Anim Sci. 1995 Jun;73(6):1839-51.
22. Lee CK., Kim SH., Moon DH., Kim KH., Son BC., Kim DH., Effects of bisphenol A on the placental function and reproduction in rats. 2005. J Prev Med Pub Health;38:330-6.
23. Barker DJP., Bull AR., Osmond, J. Fetal and placental size and risk of hypertension in adult life.1990 ;Br Med J.301:259-62.
24. Marie CPR., Camilla MF., Nanna V., Kristn G., Jens Bollerslev, Guttorm H., Tor H., Fetal growth versus birth weight: the role of placenta versus other determinants.2012. 7(6):39324.
25. Cunningham FG., Leveno JG., Bloom SL., Hauth JC., Wenstrom KD. Implantation and embryogenesis and placental development. in: 2005. Williams obstetrics 22nd edition, New York: Mc Graw-Hill; 39-90.

257 **Table 1 Effect of Oral Administration of Aqueous Leaf Extract of**
 258 ***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
15mg/kg body weight	302.41± 5.61*	330.20± 5.38	318.56± 2.39*
30mg/kg body weight	307.08± 5.33	312.85± 4.75*	330.35±4.75
45mg/kg body weight	294.91± 5.46*	315.02± 3.22*	332.83± 5.75

259

260 Values are expressed as mean ±SD.

261 *P< 0.05 compared with control.

262

263 **Table 2 . Effects of OralAdministration of Aqueous Leafextract of**
 264 ***A.muricata* on Placental weight.**

GROUPS	PLACENTAL WEIGHT
CONTROL	0.57±0.01
15mg/kg body weight	0.50±0.01*
30mg/kg body weight	0.46±0.01*
45mg/kg body weight	0.38±0.01*

265 Values are expressed as mean ±SD

266 *P< 0.05 compared with control

267

268 **Table 3. Effect of Oraladministration of aqueous extract of**
 269 ***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
15mg/kg body weight	4.67± 0.33	4.38± 0.04*	4.88± 0.01*
30mg/kg body weight	5.0± 0.58	4.37± 0.01*	4.85± 0.01*
45mg/kg body weight	5.33± 0.67	4.19± 0.01*	4.75± 0.01*

270 Values are expressed as mean± SD

271 *P< 0.05 compared with control

272 **Table 4 . Effects of Oral Administration of Aqueous Leaf Extract of**
 273 ***A.muricata* on Placental/Birth Weight Ratio.**

GROUPS	PLACENTAL/BIRTH WEIGHT RATIO
CONTROL	0.125±0.002
15mg/kg body weight	0.114±0.002*
30mg/kg body weight	0.104±0.001*
45mg/kg body weight	0.090±0.001*

274 Values are expressed as mean ±SD.

275 *P< 0.05 compared with control.