

Effect of Aqueous Extract of *Phyllanthus amarus* Leaves on Implantation and Pregnancy in Rats

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Summary: *Phyllanthus amarus* is a medicinal plant used widely in the treatment of many diseases. It has a long tradition of use in the Hindu Ayurvedic system of medicine and it has long been used as a medicinal agent in cultures around the world. Traditionally, in the Yoruba speaking part of western Nigeria it is used in treating sterility and difficult childbirth. This experiment was therefore designed to scientifically test the effect of aqueous extract of *Phyllanthus amarus* (AEPA) leaves on implantation and pregnancy. Animals were divided into two groups of 18 rats each after pregnancy has been established. Group I received 0.2mg/100g body weight of AEPA from day 1 of pregnancy. Group II received equal volume of distilled water serving as the control. Six rats from each group were sacrificed on days 6, 8 and 19 respectively. Implantation and pregnancy were assessed. AEPA reduced the time frame for implantation in the treated rats and caused abortion of pregnant rats. Although the aqueous extract of *phyllanthus amarus* reduces the time frame for implantation, its abortifacient effect does not support the traditional claim that it can treat sterility.

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Key: *Phyllanthus amarus*, Implantation, Abortifacient.

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Manuscript accepted :April 2010

INTRODUCTION

Phyllanthus amarus is an annual herb found in shady places among other common weeds. It belongs to the family Euphorbiaceae (Spurge family). It is commonly found in countries like Nigeria, China, Sierra Leone, Philippines, Cuba, Guam, Congo Brazzaville, central and southern India and other tropical regions. It is often seen as weed on uncultivated land. *Phyllanthus amarus* is commonly called “dobisowo” or “ehin olobe” or “ehin olubi sowo” in Yoruba part of Nigeria and “ngwu” by the Igbo tribe and “buchi oro” by the Asaba people, all in Nigeria (Adjahloun and Ake Assi 1972). It has a long tradition of use in the Hindu Ayurvedic system of medicine and it has long been used as a medicinal agent in cultures around the world.

Traditionally or locally the plant has been used for different purposes in Africa, which include treatment for difficult childbirth, oedema, and fever pain in Ivory Coast, cost-pain, and against diarrhoea and chronic/amoebic dysentery in Angola, in Kenya, it is used to relieve stomach pain. In Ivory Coast, it also has a lot of medicinal uses; it is used to counter coastal pain and sore-throat. In Yoruba part of Nigeria, it is used as an ingredient of “agbo” and infusion of its leaves is used for haemorrhoid. *Phyllanthus amarus* is tropically used as a poultice for skin ulcer, sores, swelling and itchiness (Bharatiya, 1992; Nadkarni, 1993). Also it is used traditionally in the treatment of jaundice, gonorrhoea, diabetes, frequent menstruation, dysmenorrhoea or menstrual pain as well as tachycardia, female sterility and difficult childbirth. (Adjahloun and Ake Assi 1972). It has been shown that the methanolic extract

of *Phyllanthus amarus* has a potential anti-oxidant activity and so inhibit lipid peroxidation as well as reduce blood glucose in alloxan diabetic rats (Raphael et al 2002, Kumar and Kuttan 2004). At the 3rd International Congress on Phytomedicine in Munich, a laboratory study reported that *phyllanthus* seems to have the ability to inhibit two of the pro-inflammatory enzymes (COX-2 and iNOS), making it potentially useful in the fight against inflammatory diseases such as arthritis (Keimer, et al 2000). The contraceptive effect of *phyllanthus* was reported by Rao and Alice 2001 when treated females cohabited with normal male mice were unable to become pregnant as their cyclicity was affected. However, the medical literature has not reported any adverse effects of *phyllanthus* related to fetal implantation and development during pregnancy.

We therefore carried out this study to investigate the effect of AEPA on implantation and pregnancy outcome and the possible mechanism of action of the extract.

MATERIALS AND METHODS

Adult female albino rats of the Wister strain weighing between 130 and 150g were used. The animals were obtained from the Animal house of the University of Ilorin and fed with pellets and given water ad-libitum. The animals were divided into 2 groups of 18 rats each. All the animals were mated and the day of appearance of spermatozoa in the vaginal smear or the presence of copulatory plug (or both) was taken as day 1 of pregnancy (Oderinde et al, 2002). Rats in group 1 receive 0.2mg/100mg body weight of the extract orally from day 4 of while rats in group II were given equal volume of distilled water starting from day 4 of pregnancy. Six rats in each of the two groups were randomly selected on day 6 of pregnancy and given 0.3ml, 0.5% Evans blue dye via the tail vein. The rats were killed 15min later; uteri were separated from fat and connective tissues and opened to ascertain the implantation sites as described by Iranloye and Owokunle (2008). Where present, the dye sites were carefully dissected out

with a scalpel blade and weighed to the nearest 0.01mg on a mettler balance. The corned out sites were counted and the number of uterine dye sites per rat recorded. Blood sample was also collected and analysed for estrogen and progesterone levels using Enzyme linked Immunosorbent Assay (ELISA). Another set of six rats from each group was sacrificed on day 8 and assessed for implantation sites while the remaining rats in each group were sacrificed on day 19.

Plant material and extraction.

The leaves of *Phyllanthus amarus* were collected within the campus of University of Ilorin. It was identified and authenticated by Prof. F.A. Oladele of Botany Department, University of Ilorin. The leaves were then dried and grinded to fine powder and extraction done using aqueous medium in soxhlet extraction. 50g of the powdered leaves were stirred into 2L of distilled water. This was then filtered and concentrated to give 20mg of the extract in 1ml.

Statistical Analysis

The mean ± S.E.M of all values was calculated and changes observed between the treatment group and the control were subjected to statistical analysis using the student t-test at the 95% confidence interval.

RESULTS

Days 6 and 8 of Pregnancy.

While Implantation sites were observed in both the control and the treated rats, on day 6 of pregnancy, the number of implantation sites was significantly higher (P<005) in the treated (6.72 ± 0.63) than the control (4.25 ± 0.28). Also the average weight of the implantation sites of the treated rats (8.75 ± 0.34) was significantly higher than that of control (4.81 ± 0.4) on day 6 of pregnancy (P<005). On day 8 of pregnancy there was no significant difference in the number of the sites and the average weight of the implantation sites in the control and the treated rats (Table 1).

Table 1.

Effect of Normal Saline (0.9%) and AEPA (0.2mg/100g body wt.) on implantation in rat (days 6 and 8 of pregnancy).

	Day 6		Day 8	
	Mean No of Dye site	Mean Weight (mg) of Dye site	Mean No Of Dye site	Mean weight (mg) Of Dye site
Control (Normal Saline)	4.25 ± 0.28	4.81 ± 0.40	7.23 ± 0.40	14.00 ± 0.14
Treated (0.2mg/100g AEPA)	6.75 ± 0.63*	8.75 ± 0.34 *	7.25 ± 0.26	14.5 ± 0.30

Each value is mean ± S.E.M. of six rats; *P < 0.05 compared with control, Student's t-test.

Table 2

Effect of Normal Saline (0.9%) and AEPA (0.2mg/100g body wt.) on Estrogen and Progesterone in rats on days 6 and 8 of pregnancy.

	Day 6		Day 8	
	Estrogen (ng/ml)	Progesterone (ng/ml)	Estrogen (ng/ml)	Progesterone (ng/ml)
Control (Normal Saline)	0.38 ± 0.13	4.8 ± 0.97	0.53 ± 0.04	6.3 ± 0.60
Treated (0.2mg/100g AEPA)	0.47 ± 0.06	5.96 ± 0.31	0.54 ± 0.06	6.0 ± 0.72

Each value is mean ± S.E.M. of six rats.

Foetal development (day 19)

The observations on foetal condition on day 19 of pregnancy are presented in Table 3. Observation of the uterus of the AEPA-treated rats on day 19 showed absence of foetus in almost all the rats (94.59% resorption) although pregnancy has been established in all the rats earlier.

In the control rats the mean foetal weight was 5.35 ± 0.06 while the percentage of viable foetus is 97.48%.

Table 3

Effect of Normal Saline (0.9%) and AEPA (0.2mg/100g body wt.) on the condition of pregnancy at day 19 of gestation.

Group	Mean fetal weight (g)	Resorption ^a (%)	Viable foetuses (%)
Control	5.35 ± 0.06	2.52	97.48
Treated (0.2mg/100g AEPA)	3.24* ± 0.11	94.59	5.41

^aResorption is used in this study as defined by Elegbe et al 1978 to mean any conceptus regardless of its age in which development has stopped and degenerative stages are visible.

DISCUSSION

The results of the study showed that Phyllanthus amarus hastened implantation but has adverse effect on pregnancy. Blastocyst implantation normally takes place in small rodents like rats on day 5 to 6 of pregnancy and it involves a basic hormonal sequence compose of a 48hour period of progesterone preparation and the presence of estrogen along with progesterone at the end of this period (Yallampalli et al 1990). For this study, implantation of the blastocyst was unhindered but rather hastened. The

time frame for implantation was reduced. The extract does not have any significant influence on the estrogen progesterone ratio within those first few days of pregnancy. Although there was no significant difference in the hormone ratio on day 6 in both the control and treated, the hormone ratio in the AEPA treated rats on day 6 was similar to that of day 8 in the control. This is suggesting that the extract hasten the release of estrogen and progesterone thus making the process of implantation faster. However this pregnancy was not sustained in the treated rats as abortion must have taken place shortly after the 8th day of pregnancy. Thus the extract may be acting on the uterine muscles directly or indirectly to cause abortion. Aqueous leave extract of Phyllanthus amarus has been found to contain some bioactive constituents such a saponins, flavonoids, tamins, alkaloids, sugar and vitamins (E & A), (Harbone, 1973). Flavonoids have been shown to have an anti-spasmodic action (Robaki et al 1988). It is therefore possible that the extract act directly on the uterine muscle to cause foetal abortion. Further study has been initiated in this laboratory to determine the effect of this extract on uterine muscle. Also the presence of tannin known to cause malabsorption and nutritional deficit (Akpantah, et al 2003) may explain the observed reduction in fetal weight in the phyllanthus treated rats compared to the control.

In summary, this study showed that aqueous extract of Phyllanthus amarus hasten implantation but does not sustain the resulting pregnancy as it causes abortion. Thus the extract may have an abortifacient property which does not support the traditional claim that it can treat sterility but may ease difficult child birth.

REFERENCES

Adjahloun and Ake Assi Useful plants of West Africa. In: H.M. Biokill. (Eds.), 1972 vol 2. pp 119
 Akpantah A.O, Oremosu AA, Ajala MO, Noronha CC and Okalawon (2003): The effect of crude

- extract of *Garcinia Kola* seed on the histology and! Hormonal! Milieu of male Sprague Dawley Rats' reproductive organs, Nigeria Journal of health and biomedical sciences. Vol. 2, N01, 40-46
- Bharatiya V.B. Selected medicinal plants of India. Bombay. Tata press, 1992 pp.235-237.
- Elegbe R.A., Amure B.O, Adekunle A.A. 1978 Induction of implantation by Aflatoxin B in the rat. Biochem Exp Biol.; 24 (2): 160-166.
- Harbone. J.B. Bioactive Constituents of plants. A guide to modern tech. of plant analysis 1973 pp. 46 -201.
- Iranloye B and Owokunle B (2008): Effect of *Garcinia kola* seed extract on female reproductive functions in rats. Int. J. Pharmacol. 4: 276 -281
- Keimer AK, Huber C, Baron A, et al 2000. Anti-inflammatory potential of *Phyllanthus amarus* inhibition of iNOS and COX-2 in murine macrophages and in isolated rat Kupffer cells. In: Farnsworth NR, Wagner H, Asakama Y, eds. Supplement II: 3rd International Congress on Phytomedicine, October 11 - 13, 2000. Munich: Nature Publishing Group; 2000.
- Kumar KB, Kuttan R. Protective effect of an extract of *Phyllanthus amarus* against radiation-induced damage in mice. J Radiat Res (Tokyo). 2004;45(1):133-9.
- Nadkarni, K. M. 1993 India material medica, vol. 1. Bombay popular prakashan private Ltd. Pp. 947-948.
- Oderinde, O., Noronha, C., Oremosu, A., Kusemiju, T. and Okanlawon, A. (2002) Abortifacient properties of Aqueous extract of *Carica papaya* (Linn) seed on female Sprague- Dawley rat. *The Nigerian Postgraduate medical Journal*. 9 (2): 95-98.
- Rao MV, Alice KM. Contraceptive effects of *Phyllanthus amarus* in female mice. *Phytother Res*. 2001;15(3):265-7.
- Raphael, K.R., Sabu, M.C., Kuttan, R. (2002) Hypoglycaemic effect of methanol extract of *Phyllanthus amarus* Schum & Thonn on alloxan-induced diabetes mellitus in rats and its relation with antioxidant potential. *Indian J Exp Biol*; 40(8), 905-909.
- Robaki, Glyglewski R.J. Flavonoids are scavengers of superoxide anion. *Biochem. Pharmacol*. 1988 37, 837-841
- Yallampalli Chandrasekhar, David T, Armstrong and Thomas G. Kennedy, Implantation delay and anti-deciduogenic activity in the rat by the anti androgen, hydroxyflutamide. *Bio. Of Reproduction*. 1990 42, pp 120 - 125