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Research Article

Abortifacient Effects of Aqueous Leaf Extract of *Annona senegalensis* Pers in Albino Rats

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Abstract

The use of abortifacient plants is commonly practiced among the natives in Nigeria as an aborting agent of pregnancy. Abortifacient substances are either chemical or physical capable of inducing abortion. One of the plants that is being used is *Annona senegalensis*, although it is primarily used as food. Its abortifacient activity is not scientifically validated. This study investigated the abortifacient activity of the leaf extract. The aqueous leaf extract of *Annona senegalensis* pers was screened for abortifacient activity in thirty (30) female albino rats at the doses of 100, 200, 300 and 400 mg/kg daily. The oral administration of the extract to female albino rats for three weeks of the gestation period resulted in reduced individual implantation sites, live fetuses, postimplantation loss and progressive decrease in serum progesterone concentration in a dose dependent manner. *Annona senegalensis* leaf therefore, has significant abortifacient activity.

Key words: *Annona senegalensis* pers, antifertility, female albino rats, abortifacient

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Introduction

There is a great population explosion in the world of a recent and is one of the greatest dilemma being faced in the world today. This has resulted in several consequences among which is a high competition over employment, education, housing, health facilities, economy, farm lands and or environment. Current global population is estimated to be over seven (7) billion and each day is on the increases. Thus, fertility control has become a global concern (Singh *et al*., 2013). Therefore, the global quest for

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antifertility agents as a substitute to resolve population explosion is of great interest, especially in developing countries.

Nigeria is one of the developing countries who is experiencing a great population explosion recently. Therefore, the search for contraceptive agents that can regulate fertility has been in existence long before now (Yadav and Jain, 2009). Induced abortion may be desired during a normal or abnormal pregnancy. Nowadays there are many synthetic preparations that are used as contraceptive agents. However, their use is limited due to their excessive side effects. In view of that, herbal medicine which is a major component of the native's traditional medicine, has attracted the attention of the World Health Organization (WHO, 1997). Based on the assessment of the health care systems in developing countries, the World Health Organization now suggested that indigenous medicinal plants could be used instead of synthetic drugs to reduce the rate of dependence on modern drugs. Nevertheless, there is a need for scientific validation of their efficacy and use.

Herbal contraceptives are in high demand due to the fact that they are cost effective, indigenously available with little side effects (Abu *et al*., 2012). Some studies have shown that many plants used traditionally as contraceptive agents are abortifacient (James *et al*., 2011), spermicidal (Abu *et al*., 2012), decreased implantation sites (Vohara *et al*., 1969), decreased estrous cycle (Koneri *et al*., 2007 and Oluyemi *et al*., 2007).

Annona senegalensis belongs to the family Annonaceae, genus *Annona* and species *senegalensis*. It is one of the species of flowering plants. The specific epithet, *senegalensis*, translates to mean "of Senegal", the country where the type specimen was first collected (Orwa *et al*., 2009). The Genus *Annona* is from the Latin word 'anon', meaning 'yearly produce' (annual harvest) referring to the production habits of fruits of the various species in this genus (Pinto, 2005; Orwa *et al*., 2009).This plant is also called African custard apple and wild sour (common names), Gwandan da'aji (Hausa), dukuuhi (Fulfulde), Hibwa (Bura), Abo (Yoruba), Uburu ocha (Ibo) and Ikpokpo (Idoma), French (pomme cannelle du Senegal, annone) and Arabic (gishta, gishta gaba) (Pinto, 2005; Orwa *et al*., 2009; Okoli *et al*., 2010; Mustapha, 2013).

The annonas are shrubs or small trees whose height varies from 5-11m depending on several factors such as species, climate, soil and crop management. They are erect or somewhat spreading in habit, with grey-brown bark, often rough and corrugated (Pinto, 2005). Annona stems are generally, ferruginous to grayish and tomentose when young, but later becoming glabrous. With few exceptions, annonas are deciduous. The system has thin lateral roots and a taproot that is not as strong as in other tropical fruit trees such as mango (*Mangifera indica* L). The lighter the soil texture, the longer the taproot grows. The taproot of a soursop tree can reach approximately 1.5-1.8m in depth (Pinto, 2005).

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Natives in Africa use this plant primarily for food. Its fruits are rich in nutrient and thereby, boost food security, foster rural development and support sustainable land care (Anon, 2014). It tends to grow better in semiarid to sub-humid regions adjacent to the coast, often, but not exclusively, on coral-based rocks with mostly sandy and loamy soils (Anon, 2014).

Generally, the annonas grow at a range of altitudes, and those with the widest adaptation to altitude are also those with the widest adaptation to latitude. No photoperiod responses have been reported (Cordeiro and Pinto, 2005). Most annonas do not adapt to low temperature, however highland species such as Cherimoya, wild soursop and to some extent custard apple are better adapted to cold weather than the lowland soursop and sugar apple (Cordeiro and Pinto, 2005). Wild soursop is adapted to various altitudes being cultivated from 0-1,800 m in Kenya and from 0-2,400 m in other parts of East Africa (FAO, 1983; Anon, 2014). It appears to have adaptation within the range of very low to moderately high rainfall regimes occurring in areas with 600-2,500 mm, while across Africa requirements are far more than 600 mm annual rainfall. It can withstand a relative humidity as low as 44% at midday. The best mean temperatures for wild soursop growth are between 16^{o}C and 30^{o}C (FAO, 1983; Anon, 2014). It is often a solitary plant within woodland savannah understory, swamp forests, river banks and former crop land left fallow for an extended period (Anon, 2014).

The flowers, leaves and fruit are edible and culinary, white fruit pulp has a mild, pineapple-like flavour. Wild Soursop fruits are sold in local markets in Africa. The fruit has a pineapple-like odour and sweet taste (FAO, 1983). It keeps for only a few days. It is used in sherbets, ice creams and for making drinks (FAO, 1988). The flower is added to spice or garnish meals; leaves are eaten by humans as vegetables, or browsed by livestock (Anon, 2014). The leaves are also used to create a general health tonic in the treatment of pneumonia, diseases of the eye, stomach and intestines (Cordeiro *et al*., 2005). The leaves have essential oils with parasiticide, antidiarrhoea, rheumatological and antineuralgic properties (Cordeiro *et al*., 2005). Boiled water infusions of the leaves have anti-spasmodic, astringent and gastric properties (Calzavara and Miiller, 1987; Khan *et al*., 1997), help in treatment of diabetes and gastric upsets (Calzavara and Miiller, 1987) and are used in kidney ailments (Cordeiro *et al*., 2005). The cooked flowers and petals are used for healing eye inflammations; the treatment requires 2-3 washes a day (Calzavara and Miiller, 1987).

The present study investigated the effect of the aqueous leaf extract of *Annona senegalensis* on abortifacient activity and the hormone, progesterone.

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Materials and methods

Collection and identification of the plant sample

The leaves of *Annona senegalensis* were collected from Girei Local Government Area of Adamawa State in July 2014. The leaves were identified and authenticated by a taxonomist in the Department of Forestry and Wild Life Management of the Modibbo Adama University of Technology Yola. Voucher specimen (PG/15/CHM/008) was deposited in the Departmental herbarium. The leaves of the plant were dried in a shade, ground with pestle and mortar into powder, and kept in refrigerator until required.

Plant extraction

The *Annona senegalensis* powdered leaf weighing 200g was used. The aqueous leaf extract was obtained using soxhlet extractor and distilled water as the solvent at about 90^{o}C. The extract was evaporated to near dryness on a water bath at 40^{o}C, and allowed to dry at room temperature and then ground into powder using pestle and mortar, weighed and yielded 63.2% and kept at 4°C in refrigerator until further use (Evans, 2009; Zade and Dabhadkar, 2013).

Experimental Animals

Female albino rats were obtained from Laboratory Animal House of the Biochemistry Department University of Maiduguri and kept in the Postgraduate Veterinary Anatomy Research Laboratory University of Maiduguri. They were given pelletized growers mash 120 g daily (Vital feeds Nigeria Ltd) and water *ad libitum*. A total of 30 rats were used for the study.

Experimental Design

Thirty (30) female albino rats weighing between 175 – 200 g were used. They were divided into five groups (1-5) of 6 rats and each female rat was cohabited (1:1) nightly with a male rat and vaginal smears observed following day. Female rats were considered pregnant if the vaginal smear observed under the high powered microscope each morning following cohabitation contained sperm or if a vaginal plug was present. Day 0 was defined as the day on which evidence of mating was observed. On day 0 each mated female rat was assigned to an experimental group at random. The rats in groups 2-5 were treated with 100 mg/kg, 200 mg/kg, 300 mg/kg and 400 mg/kg of the aqueous extract respectively. Group 1 served as control and was administered normal clean tap water. They were observed twice daily for clinical signs, mortality or adverse reactions. Detailed physical examination was performed on all the rats throughout the gestation period. Two female rats were humanely sacrificed and macroscopic postmortem examination was performed on each sacrificed rat on days 7, 14 and 20 of gestation. The uterus was carefully examined for sites of implantation and resorption/abortion as described by {William (2013). Each week, serum was harvested for progesterone assay using standard protocol according to the manufacturers' instruction. The progesterone

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assay was done using Progesterone Enzyme Immunoassay (EIA) Test Kit (10004 Perfemed Group, Inc Beijing China).

Statistical analysis

Data obtained during this study were presented as Means ± Standard Deviation (S.D). The differences among groups were analyzed by one-way ANOVA using GraphPad InStat version 3.0 (2003) computer software. P<0.05 was considered significant.

Result

The effect of aqueous leaf extract of *Annona senegalensis* on progesterone is presented in Table 1. There were significant (P<0.05) decreases in a dose dependent manner in all the treatment groups throughout the study period as compared to the control.

The effect of aqueous leaf extract of *Annona senegalensis* on implantation in female albino rats is presented in Table 2. There were implantations in all the treatment groups (100, 200, 300 and 400 mg/kg) and the control in week 1. Postimplantation loss (resorption of foetuses) were observed in week 2 in all the treatment groups at the rate of 100 % except in group 5 of the 400 mg/kg dose treatment where the rate was 90.91 % and had 5 viable fetuses.

Table 1: Effect of Aqueous Leaf Extract of *Annona senegalensis* on Progesterone in Albino Rats

Extract dose (mg/kg)	Weeks of Aqueous Extract Administration		
	1	2	3
	Mean ± SD ($X10^9$/L)		
Control	2.74±0.089[a]	2.68±0.179[a]	0.26±0.011[a]
100	2.54±0.089[c]	2.24±0.241c	0.23±0.017[c]
200	1.90±0.000[c]	1.54±0.167[c]	0.14±0.011[c]
300	1.74±0.089[c]	1.62±0.110[c]	0.15±0.017[c]
400	1.22±0.084[c]	1.80±0.100[c]	0.13±0.009[c]

Different superscripts in the same column are significantly different at P<0.05.
Mg/kg: milligram per kilogram

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Table 2: Effect of aqueous leaf extract of *Annona senegalensis* on implantation in female albino rats

Group	Treatment Dose (mg/kg)	NPR	NISIR	NRF	NVF	PL (%)
Control	-	6 (100)	(10, 11, 9, 12, 10,12)	00	64	0
2	100	6 (100)	(9,8,7,9,10,11)	54	00	100
3	200	6 (100)	(11,8,9,10,7,9)	54	00	100
4	300	6 (100)	(9,7.10,8,7,10)	51	00	100
5	400	6 (100)	(10,7,9,8,9,12)	55	05	90.91

Key: No. of Pregnant rats = NPR; No. of Implantation Sites Individual Rats = NISIR; No. of Resorption of Fetuses = NRF; No. of Viable Fetuses = NVF; Postimplantation Loss = PL Fertility index (No. of pregnant rats) % = No. of pregnant animals/No. of mated animals X 100, Postimplantation loss (resorption of fetuses) % = Total No. of implantation – Total No. of viable fetuses/Total No. of implantation X 100

Discussion

The effect of the aqueous leaf extract of *A. senegalensis* on progesterone in albino rats significantly ($P<0.05$) decreased dose dependently and progressively throughout the gestation period. This decrease may be due to the activity of flavonoids on hypothalamus-pituitary gland-gonadal axis as reported by Butterweck *et al*. (2004); Rabbani *et al*. (2005); Lobat *et al*. (2012).

Flavonoids have an important role in the functional regulation of hypothalamus pituitary adrenal axis. Since ovary and uterine functions are controlled by hypothalamus-pituitary gland-gonadal axis and their discharged hormones, tissue effects and harmonic changes are possible. Based on the direct and indirect effects of flavonoids on the nervous system and neurotransmitters via receptor sensitivity and its regulation, it is possible that progesterone decreased due to receptor desensitization in corpus luteum (Rabbani *et al*., 2005; Butterweck *et al*., 2004).

The most common causes of female infertility are hormones. These are commonly associated with ovulation, ovarian syndrome, premature ovarian failure, damage to the Fallopian tube or uterus, or problem with the cervix (Egba *et al*., 2014). Endocrine disorders result from excessive production of hormones, or insufficient production of one or more hormones or the lack of the tissues responses to normal circulating hormones (Baird *et al*., 1976). The female reproductive cycles function primarily by the interplay between the luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, estradiol, prolactin and testosterone. Also, the integrity of the female

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reproductive organs can be assayed by the serum level of these hormones (Bowman and Rand, 1985).

In this study, the extract did not inhibit implantation totally (Table 2) but decreased the serum progesterone level (Table 1) consistently throughout the gestation period. The postimplantation loss (resorption of foetuses) {Table 2} observed might be due to the progressive decrease in the progesterone concentration which was not sufficient to maintain the pregnancy. Thus abortion might be due to the activity of flavonoids on the nervous system because flavonoid is an antispasmodic agent, thus its activity couldn't have been directly on the uterus if it were so, there would not have been abortion. The possible mechanism of action of this plant that resulted into abortion may be due to the action of the extract on the hypothalamus pituitary gland gonadal axis due to the presence of flavonoids which could either be directly or indirectly. This might be true since the ovaries and the uterine functions are being controlled by hypothalamus pituitary gland gonadal axis as reported by Butterweck *et al*. (2004); Rabbani *et al*. (2005); Lobat *et al*. (2012).

Conclusion

In conclusion, *Annona senegalensis* aqueous leaf extract reduced progesterone level, reduced implantation sites, caused resorption of fetuses and implantation losses and therefore has abortifacient activities. This finding supports the traditional use of the plant as an abortifacient by the natives.

Ethical consideration
The study was approved by the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria, Faculty Postgraduate Board Ethics Committee.

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