

## Full Length Research Paper

## Effects of combinations of ethanolic extracts of *Blighia sapida* and *Xylopia aethiopica* on progesterone, estradiol and lipid profile levels in pregnant rabbits

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The effects of ethanolic extracts of *Xylopia aethiopica* fruits and *Blighia sapida* roots, used by some rural dwellers in western part of Nigeria for abortifacient purposes, were investigated using pregnant rabbits. A combination of *X. aethiopica* and *B. sapida* in a ratio 1:1 at concentrations of 10.0 mg/ kg body weight (group B) and 50.0 mg/ kg body weight (group C), and *X. aethiopica* alone at 50.0 mg/kg of body weight (group D) were administered by gastric intubations for a period of 14 days from the 12<sup>th</sup> to 25<sup>th</sup> day of gestation after which they were fasted for 18 h. Serum levels of reproductive hormones (progesterone and estradiol) and lipids (triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol) were determined at the end of the treatment. Results showed significant reductions ( $p < 0.05$ ) in progesterone and estradiol levels, significant elevations of triglycerides ( $p < 0.05$ ) and no statistically significant differences ( $p < 0.05$ ) in the levels of total cholesterol in all the treated groups compared with the control (group A). There was a statistically significant reduction ( $p < 0.05$ ) in the levels of HDL-cholesterol in Group B, a significant increase in group C and no significant reduction in group D compared with the control (group A). The levels of LDL-cholesterol in all the treated groups did not differ significantly ( $p < 0.05$ ) from the control (group A). The results suggest that ethanolic extracts of *X. aethiopica* alone or in combination with *B. sapida* may actually lead to termination of pregnancy due to reduction of progesterone and estradiol. The results also suggest that there is increased risk of hypertriglyceridemia in the users of these plants but this may not predispose them to arteriosclerosis because of the insignificant differences in the levels of LDL-cholesterol in all the treated groups compared with the control.

**Key words:** *Xylopia aethiopica*, *Blighia sapida*, ethanolic extracts.

### INTRODUCTION

Traditional medicine is used for the control of fertility by the use of anti-fertility herbs. For example, *Achyranthes aspera* have been shown to possess anti-fertility properties.

The mode of action of such plants could be through the action of hypothalamo-pituitary-gonadal axis or by direct hormonal effects on the reproductive organs which

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eventually results in the inhibition of the ovarian steroidogenesis (Shibeshi et al., 2006).

Xenobiotic metabolism in pregnancy could result in beneficial or injurious effect. For example certain medicinal plants can provoke malfunctioning of the fetus, or result in abortion (George, 1999).

*Blighia sapida* in combination with *Xylopia aethiopica* is used in the western part of Nigeria by some rural dwellers to terminate unwanted pregnancies (Abolaji et al., 2007).

The hormone progesterone, secreted by the corpus luteum and the placenta induces increased secretion by the endometrium as well as developing the glycogen and lipid stores. It also inhibits contraction of the uterine smooth muscle layer thereby preventing expulsion of the embryo. In these ways, progesterone prepares the uterus for the implantation of the fertilized ovum and its early growth (Robinson, 1978). Between the second and third months of gestation, the secretion of progesterone is taken over by the placenta (Robinson, 1978).

*X. aethiopica* is an evergreen, aromatic tree; growing up to 20m high with a clear straight bole to 75cm girth (Burkill, 1985). It is native to the lowland and the moist fringe forests in the savanna zones of Africa. The fruit of *X. aethiopica* (Grains of Selim) is used as a soup condiment and a cough remedy. *X. aethiopica* is known as Eeru (Yoruba); Uda (Igbo) and African pepper (English). A fruit extract or decoction of the bark as of the fruit is useful in the treatment of bronchitis and dysenteric conditions. It is used in Congo for the treatment of stomach aches, asthma, and rheumatism (Burkill, 1985). A decoction of leaves and roots is a general tonic in Nigeria for fever popularly called "Agbo". *X. aethiopica* has been reported to be recommended to women who have newly given birth as tonic in the Ivory Coast as a woman remedy, it is taken also to encourage fertility and for ease of childbirth (Burkill, 1985). In the Government Hospital in Ghana, *X. aethiopica* is used in combination with *Newbouldia laevis* (Bignoniaceae) for treating menstrual flow anomalies and was accordingly deemed to have abortifacient properties (Burkill, 1985). The proximate and mineral elements compositions of *X. aethiopica* have been investigated in our previous work and they were found to be rich in nutrients (Abolaji et al., 2007).

*B. sapida* belongs to the family Sapindaceae. In Nigeria, it is called Isin (Yoruba). It is an ever green tree with a dense crown. It is cultivated in India, West Indies and tropical America (Gledhill, 1972). *B. sapida* is useful in African traditional medicine. The bark pulp is used as a liniment for oedema intercostal pains in Ivory Coast. The pulp and leafy types are used as eye drops in conjunctivitis (Irvin, 1965). The ashes of the dried husks and the seeds are used in the preparation of a type of soap (Irvin, 1961). The information about the use of the root is scanty in the literature. In the western part of Nigeria, the root of *B. sapida* is used in combination with the fruit of *X. aethiopica* to terminate unwanted

pregnancy (Abolaji et al., 2007).

The nutritional potentials of the root of *B. sapida* have been investigated in our previous work (Abolaji et al., 2007). The phytochemical screening of *B. sapida* revealed the presence of steroidal alkaloids. The fruits contain saponins which are hemolytic (Dukes, 1992). The combined use of these two plant parts in the literature in relation to pregnancy state is scanty and hence this research.

## MATERIALS AND METHODS

### Plant materials

The root of *B. sapida* and the fruit of *X. aethiopica* were taken from representative portions of the plant parts. They were obtained from a commercial market at Mushin, Lagos State, Nigeria. The plant specimen was identified at Botany Department, University of Lagos, Nigeria.

### Extraction of samples

Plant materials were oven-dried at 105°C and powdered in a mortar. 100 g of the sample was subjected to soxhlet extraction in 80% v/v ethanol. The extracts were concentrated using rotary evaporator and finally dried in an open beaker for 6 h. Extracts were stored in desiccators in clean, dried glassware.

### Experimental animals

Twenty female rabbits weighing between 1.3 and 1.6 kg were used in the study. They were bred in the animal house of the College of Medicine of the University of Lagos, Idi-Araba, Lagos State, Nigeria where the study was conducted.

Rabbits obtained from the animal house of the University of Lagos, College of Medicine, Lagos State, Nigeria, were acclimatized to the environment for two weeks and maintained at room temperature (Temperature 23°C, humidity 65 to 70% 12 h dark/Light) and fed with standard commercial rabbits pellets and water *ad libitum*. The experimental design involves the administration of extracts of *B. sapida* and *X. aethiopica*, ratio 1:1, and *X. aethiopica* alone to 20 female rabbits into groups (A, B, C and D) each containing 5 rabbits per group on the basis of uniform average weight. Group A received distilled water as the control. Graded doses of 10 and 50 mg/kg body weight combinations of *B. sapida* and *X. aethiopica* (ratio 1:1) were administered to Groups B and C respectively. Group D was administered with just 50 mg/kg body weight of *X. aethiopica* alone. The administration was for a 14-day period beginning from the 12<sup>th</sup> day of gestation. At the end of treatment the animals were subjected to fasting for 18 h.

### Collection of blood and preparation of serum sample

Animals were anaesthetized at the end of the treatment period using chloroform in an enclosed chamber prior to dissection in conformity with University of Lagos animal handling ethical standards. Blood was collected by cardiac puncture into labeled sterilized drug bottles and allowed to clot at room temperature for one hour and then centrifuged at 3500 xg for 15 min. The supernatant (serum) was isolated and stored at -30°C until required for analysis.

**Table 1.** Effects of combination of *Blighia sapida* and *Xylopiya aethiopic*a on serum reproductive hormonal levels in pregnant rabbits.

Group	Dose (mg/kg body weight)	Progesterone (nmol/L)	Estradiol (pg/ml)
A	-	31 ± 0.9*	478.11 ± 0.89*
B	10	12 ± 0.3*	441.31 ± 2.5*
C	50	26 ± 0.2*	453.07 ± 4.3*
D	50	13 ± 0.8*	439.27 ± 0.70*

Values are Mean ± S.D of 5 determinations. \*p < 0.05.

**Table 2.** Effects of combination of *Blighia sapida* and *Xylopiya aethiopic*a on serum lipid profile of pregnant rabbits.

Group	Dose (mg/kg body weight)	nmol/L			
		Triglycerides	Cholesterol	HDL-cholesterol	LDL-cholesterol
A	-	0.41 ± 0.46	2.25 ± 1.77	1.70 ± 1.87	0.37 ± 0.28
B	10	1.51 ± 0.56*	2.14 ± 1.04	1.15 ± 0.4*	0.3 ± 0.36
C	50	1.82 ± 0.94*	3.88 ± 1.03	2.31 ± 1.43*	0.24 ± 0.28
D	50	1.22 ± 0.21*	2.81 ± 0.40	1.76 ± 0.78	0.50 ± 0.28

Values are Mean ± S.D of 5 determinations. \*p < 0.05.

#### Determination of lipid profile

The sera above were used to estimate total cholesterol, High Density lipoprotein (HDL) cholesterol, Low Density Lipoprotein (LDL) cholesterol and Triglyceride levels. Total cholesterol was determined by the method of Braun (1984). Triglyceride was estimated using the method of Stein and Meyers (1995). The method of Hiller (1987) was used to estimate HDL- Cholesterol while LDL-cholesterol was determined by the method of Friedewald et al. (1972).

#### Determination of progesterone and estradiol

Progesterone level was estimated using enzyme immunoassay kit method (Radwanska et al, 1978) while Estradiol was determined by enzyme immunoassay kit method (Abraham, et al, 1972).

Statistical analyses were performed with the aid of SPSS for windows (release 10.0). Group comparison was done using the analysis of variance (ANOVA) and the Student's t-test. P < 0.05 was considered statistically significant.

## RESULT

The results of the effects of combination of ethanolic extracts of *B. sapida* and *X. aethiopic*a on reproductive hormonal levels and lipid profile after 14 days of treatment are summarized in Tables 1 and 2.

The result showed a significant reduction p < 0.05 of progesterone and estradiol levels at doses of 10 and 50 mg/kg body weights of *B. sapida* and *X. aethiopic*a compared with the control group A (Table 1).

Group D administered with 50 mg/kg body weight of *X. aethiopic*a alone also showed a significant reduction p < 0.05 in the levels of progesterone and estradiol compared with the control group (Table 1).

At concentrations of 10 and 50 mg/kg body weight of combination of *B. sapida* and *X. aethiopic*a (Groups B and C respectively), there were significant elevations of triglycerides p < 0.05 compared with the control group A (Table 2). Group D administered with 50 mg/kg body weight of *X. aethiopic*a alone also showed a significant elevation (p < 0.05) of triglyceride levels compared with the control group (Table 2).

There were no statistically significant differences in the levels of Total cholesterol in all the treated groups compared with the control group (Table 2).

At a dose of 10 mg/kg body weight of *B. sapida* and *X. aethiopic*a combination, Group B, there was a statistically significant reduction p < 0.05 in the levels of HDL-cholesterol compared with the control group (Table 2). But at a concentration of 50 mg/kg body weight of combination of *B. sapida* and *X. aethiopic*a, group C, there was a significant increase in the levels of HDL-cholesterol compared with the control group (Table 2).

There was insignificant reduction in the levels of LDL-cholesterol at doses of 10 and 50 mg/kg body weight of combination of *B. sapida* and *X. aethiopic*a compared with the control group. However at 50 mg/kg body weight of *X. aethiopic*a alone, Group D, there was insignificant elevation of LDL-cholesterol compared with the control group.

## DISCUSSION

The negative effect of toxic plants has been reported to cause embryonic death, abortion and fetal abnormalities among others (Keeler, 1972). For this effect to be

noticeable, the concentration of the plant toxins must be high enough to readily cross the placenta and present at a specific time in gestation to exert its effect on the developing fetus (Keeler, 1984).

Due to the ethno medicinal uses of the fruit of *X. aethiopica* and the root of *B. sapida* in a ratio 1:1 to terminate unwanted pregnancies, their effects on the levels of progesterone, estradiol and lipid profile in pregnant rabbits were investigated, as these parameters are important in pregnancy state. For example, the steroid hormones such as progesterone and estradiol are synthesized from a common precursor, cholesterol. Also progesterone and estradiol levels are increased during pregnancy and a reduction in their levels may result in termination of the pregnancy, for example, decrease in the levels of progesterone has been linked with abortion (Knight and Walter, 2004). The circulating progesterone in the mother is also used by the fetus to synthesize important hormones like the mineralocorticoids. A reduction in maternal progesterone levels therefore means that the fetus will be affected negatively.

Since progesterone supports pregnancy by inhibiting the contraction of the uterine smooth muscle thereby preventing abortion (Robinson, 1978), reduction in its levels after administering the plant extracts at combined doses of at 10 mg/kg body weight and 50 mg/kg body weight supports the ethno medicinal claims of the use of the plant combinations for abortifacient purpose.

This present study support the report of Burkill, (1985) where *X. aethiopica* in combination with *Newbouldia laevis* (*Bignoniaceae*) was used for increasing menstrual flow at the Government hospital in Ghana and was accordingly deemed to have abortifacient properties. Also report by Knight and Walter (2004) listed *B. sapida* among known and suspected teratogenic plants.

Apart from the effects of the extract combination on the hormones, the users of the plant are also predisposed to hypertriglyceridemia because of the significant increase in the levels of triglyceride observed in the result (Table 2). This increase however may not predispose them to arteriosclerosis since there was no significant increase in the levels of LDL cholesterol.

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