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Abortifacient Potentials of Zingiberaceae *Aframomum Melegueta* (Alligator Pepper) in Adult Female Wistar Rats

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Abstract

This study investigates the abortifacient potential of aqueous seed extract of Alligator pepper (zingiberaceae *Aframomum Melegueta*) in pregnant rats. In a bid to achieve this objective, seven female rats were paired with seven male rats of proven fertility from an initial pilot study. After pregnancy was achieved, the males were removed and female rats 1 – 5 served as the experimental group while female rats 6 and 7 served as the control group. The experimental group receives 2ml/kg single dose of alligator pepper extracts intra-peritoneal at the 4th day while the control was given 2ml/kg distill water orally. Thereafter, the weights of the animals were monitored for the next 17 days. The group fed aqueous seed extract of Alligator pepper showed weight loss in the 2nd trimester (206.40±36.77g) compared to weight in the 1st trimester (215.20±41.99g) but then gain weight again in the 3rd trimester while the control had a progressive weight gain. At the end of pregnancy, while the control produced litters, the experimental rats did not produce any litter. The results of this study showed that aqueous seed extract of Alligator pepper is abortifacient and this was noted in the 2nd trimester. There is therefore need to communicate the danger pregnant women are exposed to, when this substance is used as an ingredient in food or served during religious rites and cultural practices.

Keywords

Aframomum Melegueta, Pregnancy, Abortifacient

1. Introduction

Birth control also known as contraception or fertility control are methods or devices used to prevent pregnancy (Taliaferro et al., 2011) while the planning, provision and use of birth control is called family planning (Rang et al., 2012). Although safe sex; such as the use of female condoms, can help prevent sexually transmitted infections (Chin et al., 2012), birth control methods have been used since ancient times, but effective and safe methods only became available in the 20th century (Hanson et al., 2 012). The most effective birth control methods are intrauterine devices (IUDs), implantable contraceptives and sterilizations by means of vasectomy in males and tubal ligation in females. This is followed by a number of hormonal contraceptives including oral pills, patches, vaginal rings, and injections. On the other hand, the less effective methods include barrier methods such as condoms, diaphragms and contraceptive sponge and fertility awareness methods as well as spermicides and withdrawal by the male before ejaculation.

While highly effective, sterilization it is not reversible as against all other methods that are reversible most immediately upon stopping their use (Taliaferro et al., 2011). Emergency contraceptives can prevent pregnancy in the few days after unprotected sex. Some regard sexual abstinence as birth control, but abstinence-only sex education may increase teen pregnancies when offered without contraceptive education (DiCenso et al., 2002). However, some cultures deliberately limit access to birth control because they consider it to be morally or politically undesirable (Sitruk-Ware et al., 2007) and thus, the demand for abortion when unintended and unwanted pregnancy occurs.

Of interest is the fact that pregnant women indulge in such alligator pepper flavoured foods (like pepper soup), and the fact that granulated seeds of Alligator pepper have capacity

to terminate first trimester pregnancy in Sprague Dawley rats (Inegbenebor et al., 2009a) in a manner similar to Mifepristone –a drug currently used alone or with misoprostol to induce emergency contraception and first trimester abortion (Clark et al., 2005). Alligator pepper is a dietary spice used as food flavor enhancer in Nigeria and some other parts of the world. Alligator pepper contains an essential oil, which is volatile in nature, and is extractable by hydro-distillation from the seeds of Aframomum melegueta. Gas chromatography and gas chromatography-mass spectrometry have been used in characterizing 27 compounds, which constitute 98.6% of the essential oil (Ajaiyeoba and Ekundayo, 1999). These compounds include two sesquiterpene hydrocarbons, humelene and caryophyllene, which make up 82.6% of this volatile oil (Ajaiyeoba and Ekundayo, 1999). The oxides of humulene and caryophyllene constitute another 9% of the oil while 17 other mono and sesquiterpenes account for only one percent. Five non-terpenoids are detectable in trace amounts (< 0.2%) only (Ajaiyeoba and Ekundayo, 1999).

Worrisome is the fact that Nigerian adolescents and single women with unwanted pregnancy have resort to the use of concoction made from venom herbs, including alligator pepper for inducing abortion. Could this add up to the high maternal mortality reported in the area? Indeed, deaths have been reported in Nigerian Newspapers to occur in some of the women using herbal concoction for abortion. This study is therefore necessary to determine the effect of Alligator pepper on pregnancy. The study however is intended to study the abortifacient potential of alligator pepper using pregnant Sprague Dawley rats as a model. This study is restricted to the observation of the abortifacient effect of alligator pepper (seeds) on pregnancy as indicated by weight changes, presence or absence of litters at the end of the gestational period.

2. Materials and Methods

2.1. Pilot Study

Ten female and ten male rats of comparable weight (8 to 10 weeks old) and weighing 160-198g were obtained from the animal farm of Ambrose Alli University and transferred to the site of the experiment. They were allowed two weeks of acclimatization in and kept in standard cages in a well-ventilated room during which period they received normal rat chow and clean drinking water *ad libitum*.

After the acclimatization period, a pilot study was carried out. The ten female rats were randomly put in 10 separate cages labeled F1 – F10 and were paired with the 10 male rats. They were left to cohabit for three days, so that mating could take place. Thereafter, the males were withdrawn and were put in separate cages labeled (M1 to M10) as against the female rats group they cohabited with. The female rats were left in their maternity cages for 18-25 days and those that produce litters were used for the experiment.

2.2. Preparation of Plant (Alligator Pepper) Extract

Alligator pepper was obtained from a local market in Ekpoma, Edo state, and authenticated by a botanist in the Department of Botany, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria. It was then ground into powder with a clean grinding machine.

100mg of the grinded alligator pepper was mixed with 100ml of distilled water and was allowed to stands for 2 hours in a glass jar. The jar containing the mixture was covered and stirred every 30 minutes to enable thorough mixing. Thereafter, the mixture was filtered with a filter paper into a clean beaker.

2.3. Experimental Design

The seven female rats of proven fertility from the pilot study were used for the actual animal experiment. Each female rat was kept in a separate cage and labeled F1 – F7. However, F1 to F5 served as the experimental rats while F6 and F7 served as the control rats. Also, the seven male rats of proven fertility from the pilot study were allocated to their counterpart female rats. So that each cage contained one female rat and one male rat. They were left in their various cages for three days to allow mating to occur. After three days, the males were withdrawn from the females. The male rats were put in separate cages labeled (M1-M7) based on the female rat each male rat mated with. The female rats were left in their cages as maternity cage.

2.4. Administration of the Plant Extracts (Alligator Pepper)

Each rat received normal rat chow (10g) and water was given *ad libitum* throughout the study to the control and experimental groups. However, F1 to F5 received single intra-peritoneally administration of 2ml/kg alligator pepper extracts on the fifth day (day two after removal of male) (see table 1 for the quantity of plant extract administered). The rats in both groups were then observed in their separate maternity cages for the next 18 to 22 days.

Table 1. *Orderly sequence of mating and quantity of drug administered in the experimental group and control group.*

	Group	Weight before mating	Mating group	Drug Dose
Experimental group	F1	213g	F1 – M1	0.4ml
	F2	192g	F2 – M2	0.4ml
	F3	263g	F3 – M3	0.5ml
	F4	166g	F4 – M4	0.3ml
	F5	179g	F5 – M5	0.3ml
Control group	F6	168g	F6 – M6	0.00
	F7	187g	F7 – M7	0.00

2.5. Observation and Sample Collection During Pregnancy

Body weight was measured and recorded in all the rats before mating and daily during pregnancy. However, 7, 14 and 21 days after mating represent 1st, 2nd and 3rd trimesters respectively. All rats in each cage were also observed during the period of pregnancy. The difference in weight gain was used to determine pregnancy while no weight change or decrease in weight indicates absent of pregnancy.

2.6. Statistical Analysis

The data collected were the entered into the computer for statistical analysis. The Statistical Pack for Social Sciences (SPSS) was used to analyzed the data. One way Analysis of Varian (ANOVA) was performed at 95% confidence interval and $p>0.05$ was considered statistically significance. The results were then presented in suitable tables.

3. Results

Table 2 shows the mean body weight changes of pregnant rats treated with aqueous seed extract of Alligator pepper compared with control. Mean body weight gain was observed throughout the experiment in the control group as the week progresses. However, the group fed aqueous seed extract of Alligator pepper showed weight loss in the 2nd trimester (206.40±36.77g) compared to weight in the 1st trimester (215.20±41.99g) but then gain weight again in the 3rd trimester. Compared with the control, mean body weights were not different in the 1st trimester (1.20g difference) but differ by 27.60g in the 2nd and by 58.60g in the 3rd trimesters. However, the differences in these weights between the control and test were not significantly different ($p>0.05$).

Table 2. *Mean body weight changes in pregnant rats fed aqueous seed extract of Alligator pepper (Zingiberaceae Aframomum melegueta) compared with control.*

Groups	Weight changes in grams				
	Before mating	After mating	1st week of pregnancy	nd week of pregnancy	rd week of pregnancy
Control group	177.40±9.50	177.40±18.50	214.40±21.50	233.00±20.00	272.40±45.50
Test group	202.60±37.96	190.20±35.34	215.20±41.99	206.40±36.77	213.80±37.94

Values are mean ± Standard deviation;

Figure 1 represents the pattern of weight changes in pregnant rats fed aqueous seed extract of Alligator pepper compared with the control. It was observed that mean body weight gain begins after mating (day 2) and gets to peak at day 6 but started decreasing from day 8 through day 18. Thereafter, the mean body weight started increasing again in the experimental group.

Figure 2 compares the average mean body weight gain pattern between the control and the test group fed aqueous seed extract of Alligator pepper. The control presented a progressive increase body weight gain throughout the 21 days of pregnancy and then decreases by the 22nd day after birth. However, the test group that received aqueous seed extract of Alligator pepper presented a zig zag pattern indicating mean weight gain and weight loss. Reduction in body weight after pregnancy began from the 13th day post mating through the 18th day (see figure 1 and 2).

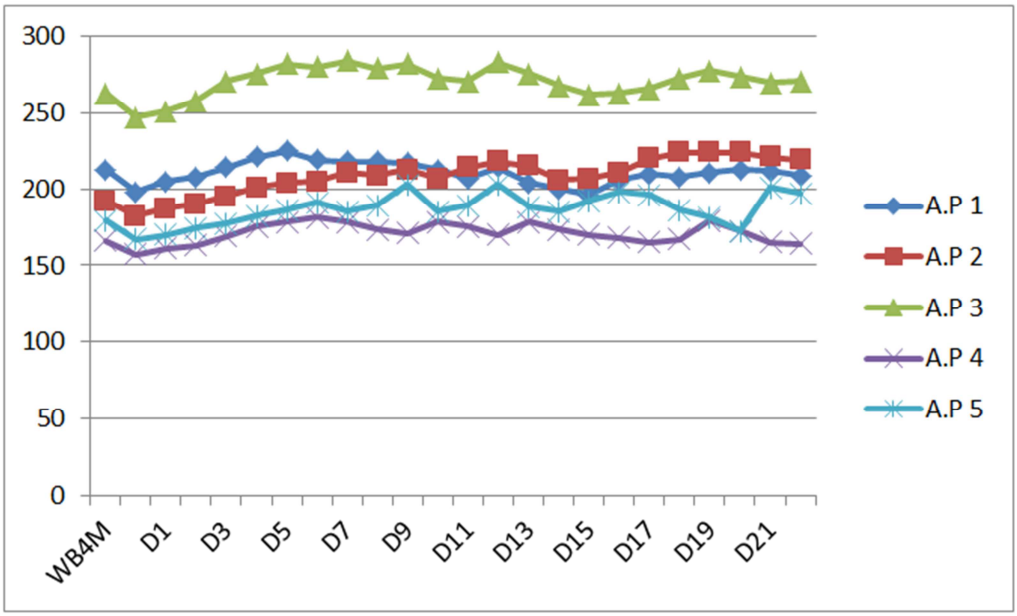

Figure 1. *Pattern of weight changes in the group fed aqueous seed extract of Alligator pepper (Zingiberaceae Aframomum Melegueta). (Key: A.P= Alligator pepper, 1 to 5 represent rat 1 to rat 5; D=day; WB4M= weight before mating).*

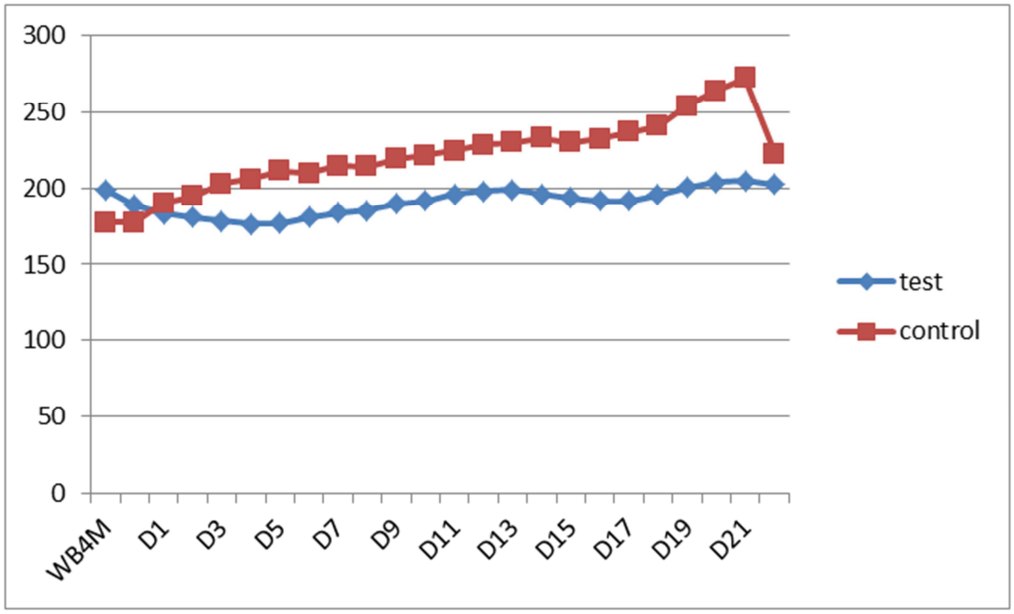

Figure 2. *Comparative average mean body weight gain pattern between the control and the group fed aqueous seed extract of Alligator pepper. (Key: Control=fed normal rat chow, test = fed aqueous seed extract of Alligator pepper, D1 to 21 number of days; WB4M= weight before mating).*

4. Discussion

This study investigates the abortifacient potential of aqueous seed extract of Alligator pepper *(*zingiberaceae *Aframomum Melegueta)* in pregnant rats. Although not statistically significant ($p>0.05$), the results showed that comparatively mean body weights gain during pregnancy between control and test *(*Zingiberaceae *Aframomum Melegueta* treated*)* groups were not different in the 1st trimester (1.20g difference) but becomes differs in the 2nd (27.60g difference) and 3rd trimesters (58.60g difference). It was also observed that the control and the test groups had similar mean body weight gain during the 1st trimester indicating the presence of pregnancy. However, mean body weight drops at the second trimester in the test and they did not litter at the end of the 3rd trimester. On the other hand, the control group maintain weight gain and produces litters at the end of the 3rd trimester.

The reduction in mean body weight in the test group at the 2nd week and the absent of litter at the end of the 3rd week indicates aqueous seed extract of Alligator pepper Zingiberaceae *Aframomum Melegueta)* is abortifacient. This findings; weight loss compared to the controls and the absence of litters by the female rats in the experimental group, contradict the usual fluid retention and weight gain during pregnancy (Denton,1982). In line with the findings of this study, Inegbenebor et al. (2009a) had previously reported alligator pepper to be abortifacient. According to Inegbenebor et al. (2009a), this could imply that the ingestion of alligator pepper by rats in his experiment does not keep or nurture pregnancy as evidenced by the fact that none of the affected experimental rats littered at the end of 21 days. In support of our finding, Inegbenebor et al. (2009b) has previously reported that granulated alligator pepper at doses above 4.0mg/kg body weight resulted in the absence of litter. It was then concluded by Inegbenebor et al. (2009b), that while not advocating abortion the dose if worked out in humans could be utilized in fertility clinics, as a safe abortifacient if the mother's life is seriously endangered by the pregnancy.

Observation of the experimental rats after the administration of Alligator pepper extract revealed increase water intake by the rats. Also observed was the presence of copious blood stained vaginal discharge. The observed copious blood stained may indicates the abortifacient capacity of the aqueous seed extract of Alligator pepper. Indeed, for many years it has been known that piperine (the active constituent of Alligator pepper) interfere with the reproductive process and Piper from different species has been used in indigenous drug preparations for inducing menstruation and terminating of early pregnancy (Johri et al., 1992). Black pepper has been reported to be used by Malay women as an abortifacient. In female mice, it has been shown that piperine effectively inhibits implantation, produces abortion and delays labor when administered during gestation and was proposed to disturb the estrogen–progesterone balance essential to maintain pregnancy (Piyachaturawat et al., 1982).

Conclusively, this study showed that aqueous seed extract of Alligator pepper *(*Zingiberaceae *Aframomum Melegueta)* is abortifacient. There is therefore need to communicate the danger, pregnant women are exposed to, when this substance are used as an ingredient in food or served during religious rites and ceremonies. Although it is unethical to further investigate this experiment in human subjects, it is however recommended that, women preparing to get pregnant and those already pregnant (specifically in their second trimester of pregnancy) avoid ingestion of Alligator pepper (either from food source or otherwise) if they are desirous of childbirth.

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