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**EFFECT OF SALINE EXTRACT OF ALLIGATOR PEPPER (*ZINGIBERACEAE*
AFRAMOMUM MELEGUETA) ON SERUM PROGESTERONE IN PREGNANT
SPRAGUE DAWLEY RATS**

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ABSTRACT

This study was done to determine the cellular basis of the abortifacient effect of Alligator pepper on first trimester pregnancy using forty five female Sprague-Dawley rats mated with male rats for three days. Thereafter, the female rats were randomly selected into three groups, A, B and C. The rats in each group were further randomly selected into three subgroups A₁, A₂, A₃; B₁, B₂, B₃; and C₁, C₂, C₃ for groups A, B and C respectively. Each rat in group A was administered with intra-peritoneal injection of 2ml of normal saline, while each rat in groups B and C was administered with 6.7mg/kg and 13.3mg/kg/body weight of saline extract of Alligator pepper on day 4 of gestation respectively. Blood was collected from A₁, B₁, C₁ on day 7; from A₂, B₂, C₂ on day 14 and from A₃, B₃, C₃ on day 21 by intra-ventricular aspiration and assayed for progesterone using ELISA technique. Results showed that progesterone level decreased significantly on days 7 and 21 of pregnancy among rats administered with 6.7mg/kg body weight, but day 7, only those treated with 13.3mg/kg /body weight of saline extract of Alligator pepper. Alligator pepper is anti-progesterone.

Keywords: Saline extract, Alligator pepper, Progesterone, Sprague Dawley Rats, Pregnancy

INTRODUCTION

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).

Of interest is the fact that pregnant women indulges in such alligator pepper flavoured foods (like pepper soup), and the fact that granulated seeds of Alligator pepper has 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). Mifepristone is in a class of medications called anti-progestational steroids (Hazra and Pore) that works by blocking the activity of progesterone (Medline Plus, 2014). This study was therefore carried out to determine if the mechanism of action of

Alligator pepper in first trimester termination of pregnancy is similar to that of mifepristone and related to progesterone antagonism.

MATERIALS AND METHODS

Research Design: This was a controlled interventional study using Sprague Dawley rat as a model.

Substance of Study: Dried pods of Alligator pepper were obtained from a local market in Ekpoma, Edo State of Nigeria. The seeds were washed, dried under the heat of the sun, and milled into powder using a China made electronic Binatone grinder.

Experimental Animals: Forty five female rats and 45 male rats of the same strain (weighing between 140 to 160g) and of proven fertility, were purchased from the animal House of Ambrose Alli University, Ekpoma, Nigeria.

Acclimatization: The experimental rats were kept in standard cages within a well ventilated laboratory and fed with grower's mash and clean water *ad libitum* for two weeks of acclimatization to the new environment. Their beddings, which consisted of saw dust, were changed daily.

Mating arrangement: Forty five male rats were randomly placed in cages containing the female rats so that each cage contained a male and a female rat. They were left in the cages for three days to enable mating to occur. To check for successful mating, the vagina was examined every morning, and vagina smears were obtained to see if sperm cells were present. In addition, the vagina and the floor of the cages were observed to check for the presence of cornified plug. The presence of sperm cells in the vagina smear or the availability of cornified plug in the vagina or on the floor of the cage indicated successful mating, and this was regarded as day-1 of gestation (Ojieh et al., 2013). Thereafter, the female rats were placed in separate maternity cages.

Grouping of the Female Rats: The female rats were randomly selected into three groups (A, B and C) so that each group consisted of 15 pregnant rats. The rats in each group were further randomly selected into three subgroups so that group A had subgroups A₁, A₂, A₃ and groups B and C had subgroups B₁, B₂, B₃ and C₁, C₂, C₃ respectively.

Preparation of saline extract of Alligator pepper: 100mg of granulated alligator pepper was mixed with 100ml of normal saline and was allowed to stand for 2 hours. The glass jar containing the mixture was covered and inverted and returned to normal position every 30 minutes to enable thorough mixing. Thereafter, the mixture was filtered with a filter paper into a clean beaker. The concentration of the filtrate was therefore 1mg of alligator pepper per 1ml of filtrate, which was labeled as the saline extract of alligator pepper.

Administration of saline extract of Alligator pepper: Each of the pregnant rats in control group A (subgroups A₁, A₂, A₃) was administered with intra-peritoneal injection of 13.3ml/Kg body weight of normal saline on day 4, a day after the separation of the female rats from the male rats. On the other hand, each of the pregnant rats in experimental group B, (subgroups B₁, B₂, B₃) and group C (subgroups C₁, C₂, C₃) was administered with intra-peritoneal injection of 6.7mg/Kg body weight and 13.3mg/Kg body weight of the saline extract of Alligator pepper respectively on same day 4 of gestation. These doses were selected in line with a previous study by Inegbenebor et al., (2009a,b).

Blood Collection and Hormonal Assay: After an overnight fast, five rats each from groups A, B, and C were euthanized by cervical dislocation under chloroform anesthesia and blood was immediately collected by ventricular puncture as described by Mojekwu et al. (2011). This was done on days 7, 14 and 21 or the rats in subgroups A₁, B₁, C₁; A₂, B₂, C₂ and A₃, B₃, C₃ respectively.

Confirmation of pregnancy Status: After the ventricular puncture and blood collection, the rats in groups A, B, and C were dissected to reveal the embryo in their gestational uterine cavities as shown in figure 1.

Hormonal Assays: The blood samples were assayed with Progesterone ELISA (Ref EIA 1561) produced by DRG Instruments GmbH, Marburg, Germany as described in ALPCO's Users manual.

Data Analysis: The results of the measurements made were cross-tabulated and means and standard deviations were calculated. The difference between the control and experimental values of serum Progesterone were observed and p

values at 5% level of significance were calculated using Daniel Soper's Statistical calculator online (DanielSoper.com, 2014).

RESULTS

The serum progesterone levels were higher in the pregnant rats treated with intra-peritoneal injection of normal saline than in pregnant rats treated with 6.7 and 13.3 mg /Kg body weight of saline extract of with Alligator pepper (See table 1). However these were significant on days 7 and 21 with the dose of 6.7mg/kg body weight and on day 7 only at a higher dose of 13.3mg/kg body weight. The serum progesterone level in rats treated with 13.3 mg/Kg body weight of Alligator pepper was significantly lower ($p=0.002$) than in rats treated with 6.7mg/Kg body weight of alligator pepper in the first trimester (Subgroup C1 vs. Subgroup B1).

Table 1: Effect of different doses of Alligator pepper on Blood Progesterone Levels in Sprague Dawley Rats on days 7, 14 and 21 of gestation

| Group | No. of Rats | Pregnancy Status | Dose of Alligator Pepper in Kg/body wt | Blood Progesterone Level \pm SD ng/ml | | |
|-------------------|-------------|------------------|--|--|--|--|
| | | | | Day 7 First trimester | Day 14 Second trimester | Day 21 Third Trimester |
| A | 15 | Pregnant | Nil | 35.08 \pm 14.37 Subgrp A ₁ | 34.95 \pm 22.03 Subgrp A ₂ | 45.6 \pm 3.58 Subgroup A ₃ |
| B | 15 | Pregnant | 6.7mg | 10.55 \pm 2.94 Subgrp B ₁ | 22.8 \pm 12.47 Subgrp B ₂ | 39.3 \pm 1.04 Subgrp B ₃ |
| P value (A and B) | | | | 0.006** | 0.314 | 0.005** |
| C | 15 | Pregnant | 13.3mg | 3.45 \pm 2.02 Subgrp C ₁ | 13.55 \pm 6.87 Subgrp C ₂ | 44.3 \pm 7.04 Subgrp C ₃ |
| P value (A and C) | | | | 0.001** | 0.072 | 0.722 |
| P value (B and C) | | | | 0.002** | 0.184 | 0.155 |

** significant decrease in progesterone



Figure 1: Sprague Dawley Rat Gestational Uterus showing multiple fetuses

DISCUSSION

Progesterone is secreted by the corpus luteum soon after ovulation and is responsible for the growth and development of the endometrium -a process which prepares the endometrium for implantation of the fertilized ovum. The normal human endometrium reacts precisely and sensitively to any hormonal stimulation with predictable changes. If the corpus luteum develops normally after ovulation, the progesterone secreted induces specific changes in endometrial glandular and stromal cells that can be dated by daily fine-structural alterations (Dallenbach-Hellweg, 1988). With corpus luteum deficiency, the endometrial differentiation is delayed and remains incomplete (Dallenbach-Hellweg, 1988).

Normal progesterone levels during a woman's menstrual cycle and during pregnancy are different. From day 1 to 14 of a menstrual cycle, the levels should be between 1 and 1.5ng/ml. From day 15 to 28, the levels vary from 2 to 28ng/ml. During the first trimester of pregnancy, normal levels can range from 9 to 47ng/ml. A minimum level of 10ng/ml during the first few weeks of pregnancy is considered necessary for a viable pregnancy (Baby 2 See.com, 2014). If a woman has low progesterone levels, it can be an indication of a threatened abortion. Second trimester levels will range from 17 to 147ng/ml and third trimester levels should be between 50 and 200ng/ml (Baby 2 See.com, 2014). In this study, progesterone level was 35.08 ± 14.37 ng/ml, 34.95 ± 22.03 ng/ml and 45.60 ± 3.58 ng/ml in the first, second and third trimesters respectively, of the non-treated pregnant rats in group B. The large variations in progesterone levels as indicated by large standard deviations are consistent with the large variations in women in all trimesters of pregnancy.

Progesterone usually increases with increasing gestational age as it was found in this study. Its special function in pregnancy is to sustain pregnancy till term. Discontinuation of progesterone in the first trimester of pregnancy is the basis of the abortifacient effect of mifepristone (Medline Plus, 2014). In this study, saline extract of Alligator pepper was found to significantly decrease progesterone level in the first trimester of pregnancy, though abortion did not occur. However, a higher dose (13.3mg/Kg body weight) of saline extract of Alligator pepper caused a significant decrease in serum progesterone level ($p=0.002$) when compared to a lower dose (6.7mg/Kg body weight) of Alligator pepper. In addition, abortion occurred in a previous study with a higher dose of 333mg/Kg body weight of Alligator pepper (Inegbenebor et al., 2009a,b). Deficiency in progesterone is probably the basis of the effect of granulated seeds of Alligator pepper on first trimester of pregnancy.

Progesterone synthesis has been shown to be hindered in rat testicles associated with tetrahydrocannabinol (Jakubovic et al., 1979). These effects are also seen with Cannabidiol and Cannabinol, which are more effective than tetrahydrocannabinol (Dalterio et al., 1978). The enzyme β -hydroxysteroid dehydrogenase converts pregnenolone formed from cholesterol to progesterone, whose synthesis is a step in the synthetic pathway of testosterone in the testis (Boron and Boulpaep, 2011). Beta Caryophyllene, a common constituent of the essential oils of numerous spices and food plants such as Alligator pepper, selectively binds to the type 2 cannabinol receptor (CB_2) ($K_i = 155 \pm 4$ nM) and is a functional type 2 cannabinol receptor CB_2 agonist (Gertsch et al., 2008). Therefore, beta-caryophyllene is the most probable active ingredient of Alligator pepper involved in inhibition of progesterone synthesis by the ovary.

Like mifepristone, Alligator pepper in high doses may also be used for emergency contraception. Excessive hemorrhage is commonly the side effect when teenagers in Nigeria use it to procure unsafe abortion (Inegbenebor, 2006; Inegbenebor, 2008). This may be prevented by combining its use with misoprostol as is done with mifepristone (Clark et al., 2005; American College of Obstetricians and Gynecologists, 2014).

Conclusion

Intraperitoneally injected Saline extract of Alligator pepper significantly reduces progesterone levels during pregnancy. Higher levels of saline extract of Alligator pepper are more effective. The mechanism of action is most probably the inhibition of progesterone synthesis by beta-caryophyllene component of the sesquiterpene constituent of Alligator pepper.

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AUTHORS' CONTRIBUTIONS

Dr Inegbenebor, U., conducted the research and wrote all aspects of this article with supervision from Dr Ebomoyi, M.