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TERATOGENIC EFFECT OF THE ETHANOLIC LEAF EXTRACT OF *Momordicafoetidaschum* (Cucurbitaceae) ON THE MORPHOLOGY OF FOETAL SPRAGUE DAWLEY RATS

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ABSTRACT

The teratogenic effects of the ethanolic leaf extract of *Momordicafoetida* on the morphology of foetal Sprague Dawley rats was studied using 30 cyclic females and 5 males Sprague Dawley rats (weighing 200-300g) for the study. Vaginal lavage was taken daily to monitor their estrous cycle for 4 weeks. On the proestrous day of the cycle of each rat, a male rat was introduced into the cage, the presence of spermatozoa in the vaginal lavage was taken as day 1 of conception. The pregnant female Sprague Dawley rats were randomly divided into six groups which include group A₁ and A₂ (control), group B₁ and B₂ (anti-implantation) and group C₁ and C₂. Treated rats in group B₁ and B₂ received a double and single oral doses of 500mg/kg and 250mg/kg bw of the extract from post-coital day 1 to 10, while the control animals (A₁) in this group received an equal volume of distilled water. All the animals were sacrificed on the day 12 of gestation to assess for anti-implantation effect. Treated rats in group C₁ and group C₂ received double and single oral doses of 500mg/kg and 250mg/kg bw of the extract from the 6th-19th day of gestation while the control animals (A₁) in this group received an equal volume of distilled water. All the animals were sacrificed on the 12th day of gestation to assess for anti-implantation effect. All pregnant rats were sacrificed, fetuses were examined for malformation and gestational parameters monitored were: number of total implantation; resorption and dead fetuses. Live fetuses were weighed and examined for external malformations and variations. Fetal parameters recorded were: fetal numbers and weight, crown rump length, trans-umbilical cord length and placental weight. The results showed that there was prevention of implantation, abortion and significant reduction of parameters: crown rump-length, trans-umbilical cord length compared to the control (P<0.05). In conclusion, the above result suggest that the ethanolic extract of *Momordicafoetida* leaf at 500mg/kg body weight prevents implantation, induces abortion and significantly reduces fetal parameters in Sprague-dawley rats.

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INTRODUCTION

The use of plant - based systems continues to play an essential role in health care. It has been estimated that approximately 80% of the population in developing countries depend on traditional medicine for their primary health care. In Africa societies, the tradition of collecting, processing and applying plant –based medications have been handed down from generation to generation. Traditional medicine, with medicinal plants as their most important component are sold in market places or prescribed by traditional healers in their homes (Karou *et al*, 2005).

Momordicafoetidaschum (cucurbitaceae) belongs to the family plantae, it is a perennial climbing vine of tropical Africa closely related to the bitter melon (*M.charantia*) and Balsam apple (*M.balsamina*). It is known as concombresauvage in french, ewe ejirin in Yoruba land, isugualu-osi, in Igbo and abos-a-wir in tsekeri.

The leaves have a bitter taste and the fruit is edible and consumed in various parts of the world. The root is also considered edible like its relative *M. charantia*. Its specie name (Bad smelling) refers to its unpleasant smell. It was previously named *Momordicamorkona* and *M. cordata* (Schaeffetal., 2010).

Different parts of *Momordicafoetidaschum* (cucurbitaceae) have various medicinal purposes, as they would be used for the treatment of sever headache, earache, fever, diabetes, indurations of the mammary glands, boils, stomach ache, intestinal disorder, worms, purgative, snake bite, dropsy, malaria, to mention but a few (Burkill 1985). In spite of its wide uses and application *Momordicafoetidaschum* (cucurbitaceae) have been reported to contain among other constituent toxic substances like cucurbitane type triterpenoid derivative, such as triterpene acids, tannin and volatile oil capable of inducing abortions, agglutination, haemolysis of the red blood cells and irreparable damage to vital organs like kidney and liver (Dulcie *etal.*, 1997).

MATERIALS AND METHODS

Plant Materials

The leaves of *MomordicaFoetidaschum* (cucurbitaceae) was harvested along Abatadu village in Ikire Township in Irewole Local Government in Osun State Nigeria and was authenticated by a botanist from the University of Lagos, with voucher number LUH 1419A.

The whole plant was thoroughly washed, rinsed in water and allowed to air dry. The dried plant samples were macerated into a fine dried powder with a domestic electric grinder and stored in an air tight glass jar 250g of macerated dried leaves was obtained.

The extraction process was performed in the pharmacognosy department of the University of Lagos where the pulverized plant was extracted with 95%wt/vol ethanol and water immediately before use.

EXPERIMENTAL DESIGN

Thirty five adult Sprague dawley rats (*Rattusnovergicus*) were obtained from the animal breeding laboratory Centre and housed in standard well ventilated metal cages, in the rat control room of the department of Anatomy, of the College of Medicine University of Lagos. 5 male and 30 female rats of proven fertility, with average weight of 200-300g were used for the study. They were kept separately (5 animals per cage) except when mating was necessary according to the experimental design. The rats were exposed to cycles of 12 hours of light and 12 hours of darkness each 24-hour period, a relative humidity of 50% to 55% and temperature range of 26⁰C to 28⁰C. The animals were fed with growers mash and clean tap water *adlibitum*. The rats were allowed to acclimatize for 4 weeks prior to the start of the experiment. Routine cleaning of the cages were done daily.

EXPERIMENTAL PROCEDURES

During one month, every morning between 8-9am vaginal lavage was taken using Pasteur pipette with 10% normal saline and smeared on microscopic slides to determine estrous cycle in female rats (Mandi, 1991).

This investigation was conducted in a series of experiments designated A through C. the extract and distilled water were administered to the treated and control animals respectively by gastric lavage.

Experiment A (Anti-implantation effect of *MomordicaFoetida*) schum (cucurbitaceae) Ten female and 5 male rats were used in this experiment. The female rats were randomly divided into 2 groups (B₁ and B₂) of 5 rats each, comprising the high dose (B₁) and the low dose (B₂). Each female rat was placed in the cage of a proven male breeder between 2pm and 4pm during proestrus and was left with the male rat until 10am the following day. Each female rat was examined on the day of estrous, and the presence of vaginal plugs or the presence of sperm in the vaginal smear was used as evidence that an animal had mated. Each rat in group B₁ received 500mg/kg of body weight of the extract from postcoital day 1 to 10 and each rat in group B₂ also received 250mg/kg of body weight of the extract from postcoital day 1 to 10 to observe for possible anti-implantation effect. Whereas the control group A₁ received an equal volume of distilled water.

Experiment B (Effect of *MomordicaFoetidaschum* (cucurbitaceae) on the fetus).

Ten female and 5 male rats were used in this experiment. The female rats were randomly divided into 2 groups (C₁ and C₂) of 5 rats each. Each female rat was placed in the cage of a male rat of proven fertility between 2pm and 4pm during proestrus and left with the male rat until 10 Am the following day. Each female rat was examined on the day of estrous, and the presence of a vaginal plug or the presence of sperm in the vaginal smear was used as evidence that an animal had mated. The animals in group C₁ received 500mg/kg of body weight of the extract from the 6th to 19th days of gestation and, each rat in group C₂ also received 250mg/kg of body weight of the extract from the 6th to 19th days of gestation where as the control group A₂ received an equal volume of distilled water. Body weight, food consumption, gross appearance and behavioural pattern of pregnant Sprague dawley rats were monitored daily.

COLLECTION OF FETUSES

On the 12th and 19th day of gestation, rats at the time of sacrifice were weighed and then anaesthetized by chloroform inhalation. Fetuses were recovered from pregnant rats by ventral laparotomy and examined. The number of fetal implants, resorptions, live and dead fetuses was recorded. Live fetuses were removed from the uterus, weighed and examined for gross malformations. Fetal parameters such as fetal numbers, trans-umbilical length, tail length, crown-rump-length and placental weight were measured.

Experimental procedures involving the animals and their care was conducted in conformity with International and Institutional Guide Lines for the care of laboratory animals in biomedical research and also the animal experimental model used was in conformity to the guiding principles for research involving animals as recommended by the declaration of Helsinki and the guiding principles in the care and use of Animals (2007).

STATISTICAL ANALYSIS

The data analysis for this research was reported as mean \pm standard deviation. Statistical analysis was performed by using one way analysis of variance and the scheffe post hoc test.

RESULTS

EFFECT ON THE WEIGHT OF MATERNAL SPRAGUE DAWLEY RATS

Maternal Sprague dawley rats (treated groups) showed significant body weight changes at $p < 0.05$, during and after oral administration of ethanolic leaf extract of *MomordicaFoetidaSchum* (cucurbitaceae) there was also significant changes in food consumption, behavioural pattern and gross appearance.

EXPERIMENT A (ANTI-IMPLANTATION EFFECT OF *Momordica*

foetidaschum(cucurbitaceae)

Administration of 500g/kg of body weight of ethanolic leaf extract, of *Momordicafoetidaschum* (cucurbitaceae) to rats in group B₁ on postcoital days 1 to 10 caused vaginal bleeding in rats on the 4th and 5th day of oral administration of the extract. Despite successful mating demonstrated by the presence of sperm plugs, uterine examination on postcoital day 12 revealed no implantation or resorption sites in all the animals treated with the extract. Administration of 250mg/kg of body weight of the ethanolic leaf extract of *Momordicafoetidaschum* (cucurbitaceae) to rats in B₂ on postcoital days 1-10 had no anti-implantation effect on the rats that is there was implantation in all 5 rats.

All the rats in the control group A₁ became pregnant and a mean of 10.00 implantations was recorded in the horns of the uteri of these rats during examination on postcoital day 12.

EXPERIMENT B (EFFECT OF *MomordicafoetidaSchum* (cucurbitaceae) ON THE FETUSES)

Maternal administration of 500mg/kg of body weight of ethanolic leaf extract of *Momordicafoetidaschum* (cucurbitaceae) to rats in C₁ on the 6th to 19th day of gestation caused death of 3 animals, on the 14th and 15th day of gestation which is the 7th and 8th day of administration of the extract due to severe bleeding. Obvious vaginal bleeding occurred in two rats on the 14th day of gestation which also lead to their death.

Maternal administration of 250mg/kg of body weight of ethanolic leaf extract of *Momordicafoetidaschum* (cucurbitaceae) to rats in group C₂ on the 6th to 19th day of gestation caused significant decrease in placental weight, crown-rump length, umbilical length, and fetal weight.

There were no vaginal bleeding episodes, no early deliveries and no death of animals in the control group. All fetuses were well implanted and alive.

ABORTIFACIENT EFFECTS

Due to the severe vaginal bleeding in all the rats of group C₁, lifeless fetuses were seen on the floor of the cages on the seventh and eighth day of administration, uterine examination on postcoital day 15 revealed resorption sites in the animals treated with the extract.

Table 4:Effect of ethanolic leaf extract of *Momordicafoetidaschum* (cucurbitaceae)on implantation in Sprague dawley rats fed with the extract on postcoital days 6 to 19 of gestation

Fetal variables					
Group	No of fetuses	Crown rump length (cm)	Tail length (cm)	Fetal body weight (g)	Placental weight (g)
Control	10.00 ± 0.71	4.04 ± 0.08	1.19 ± 0.04	2.70 ± 0.15	0.55 ± 0.07
Extract250mg/kg	10.00 ± 0.84	2.13 ± 0.12 ^b	0.60 ± 0.20 ^c	0.88 ± 0.11 ^c	0.40 ± 0.11
50mg/kg	10.00±0.80	1.14±0.11 ^b	0.30±0.10 ^c	0.60±0.11 ^c	0.20±0.10

LIST OF PLATES

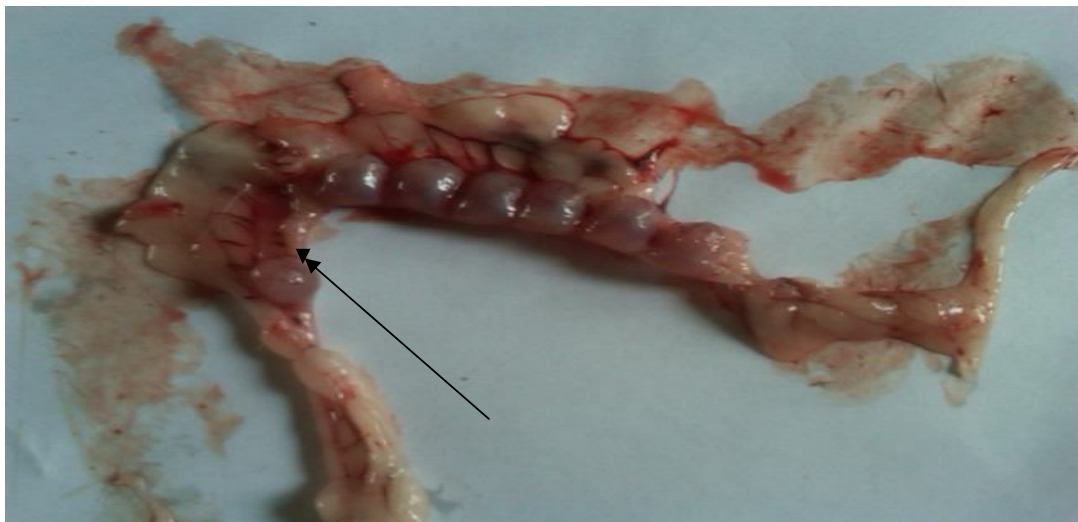


Plate1: Arrow showing the fetuses. Effect of low dose (250mg/kg – wt) of the extract on maternal Sprague dawley rat



Plate 2: Arrow showing the fetuses. Effect of low dose (250mg/kg – wt) of the extract on maternal Sprague dawley rat

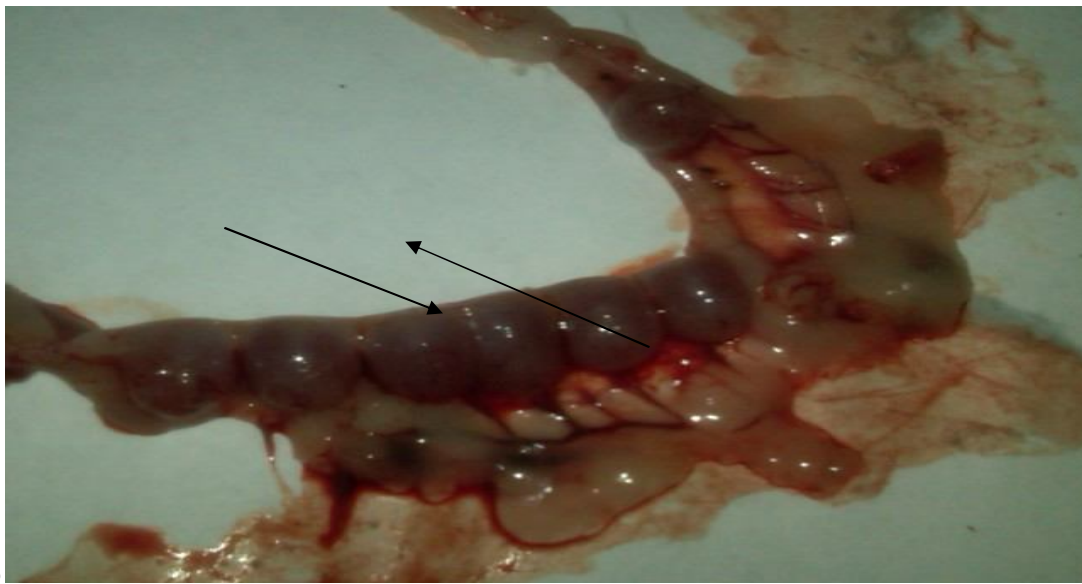


Plate3: Arrow showing the fetuses. Effect of Low Dose (250mg/kg-wt) of the Extract on Maternal Sprague Dawley Rat.

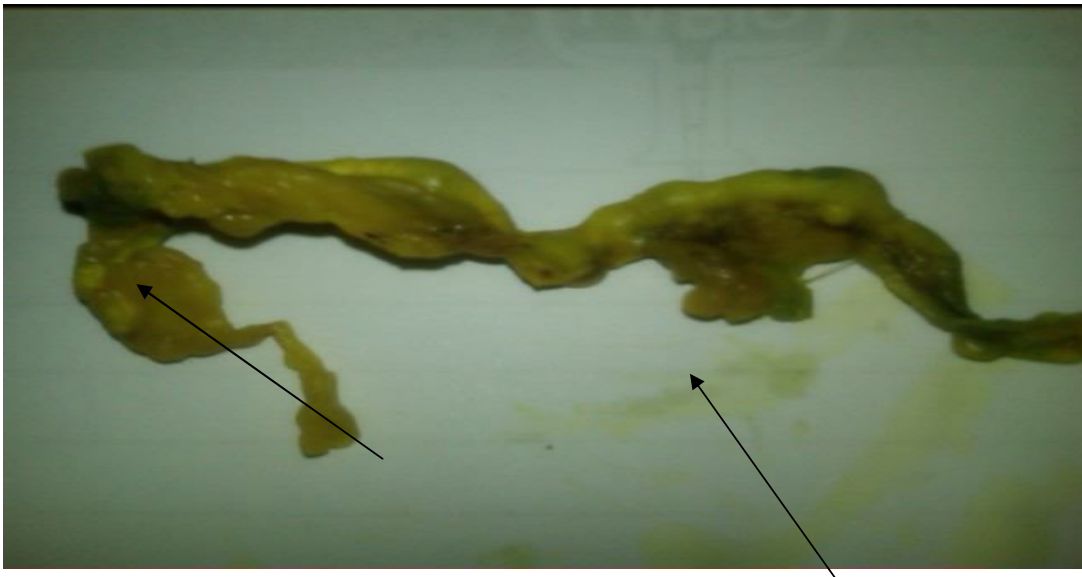


Plate 4: Arrows showing Resorption sites in maternal Rats due to the effect of the extract (500mg/kg – wt)



Plate 5: Arrows showing Resorption sites in maternal Rats due to the effect of the extract (500mg/kg – wt)

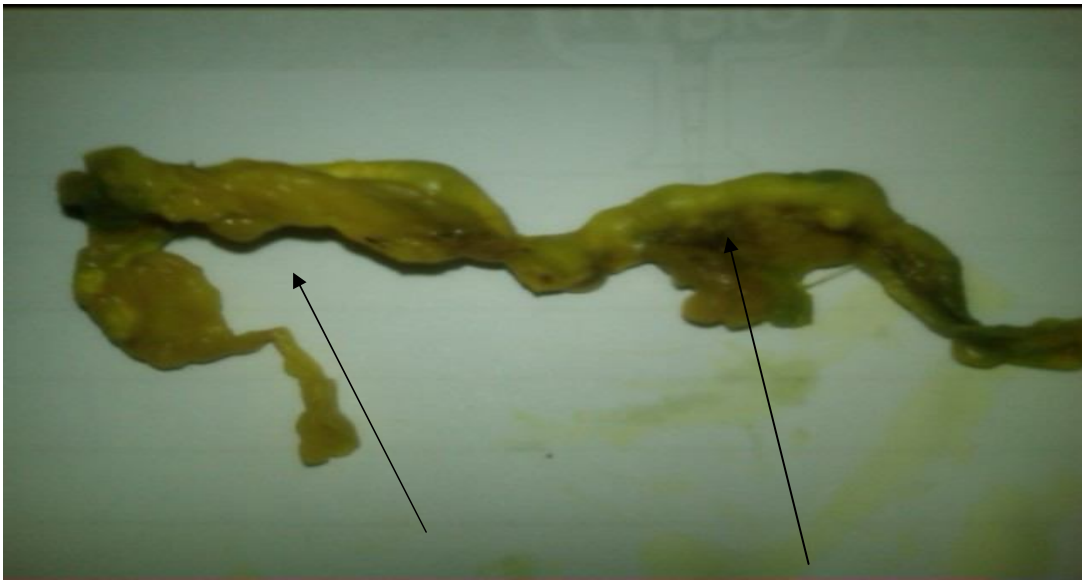


Plate 6: Arrows showing Resorptionsites in Maternal Rats due to the Effect of the Extract (500mg/kg-wt).



Plate 7: Arrow showing live fetuses recovered from maternal rats.

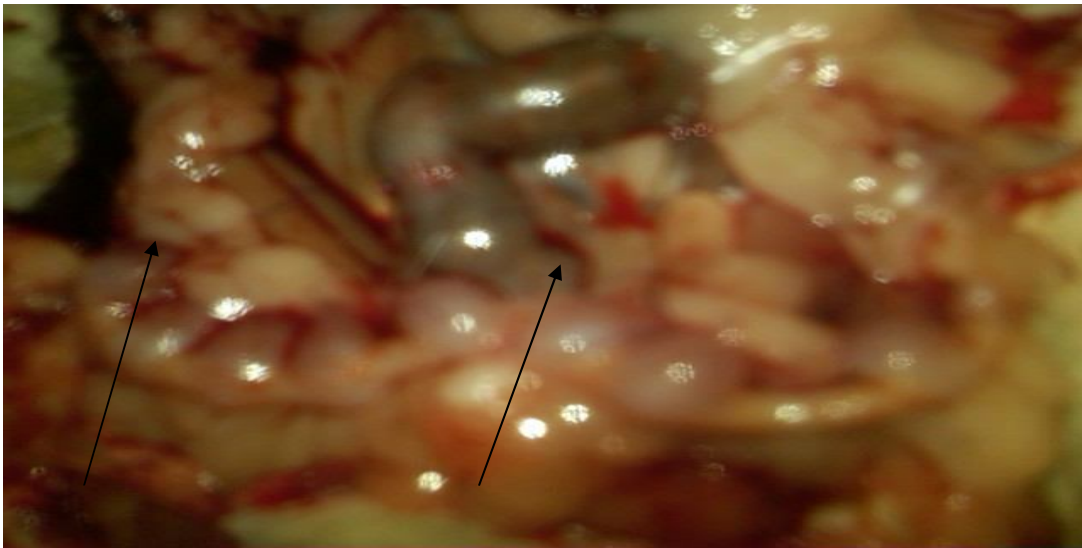


Plate 8: Arrow showing live fetuses recovered from maternal rats.

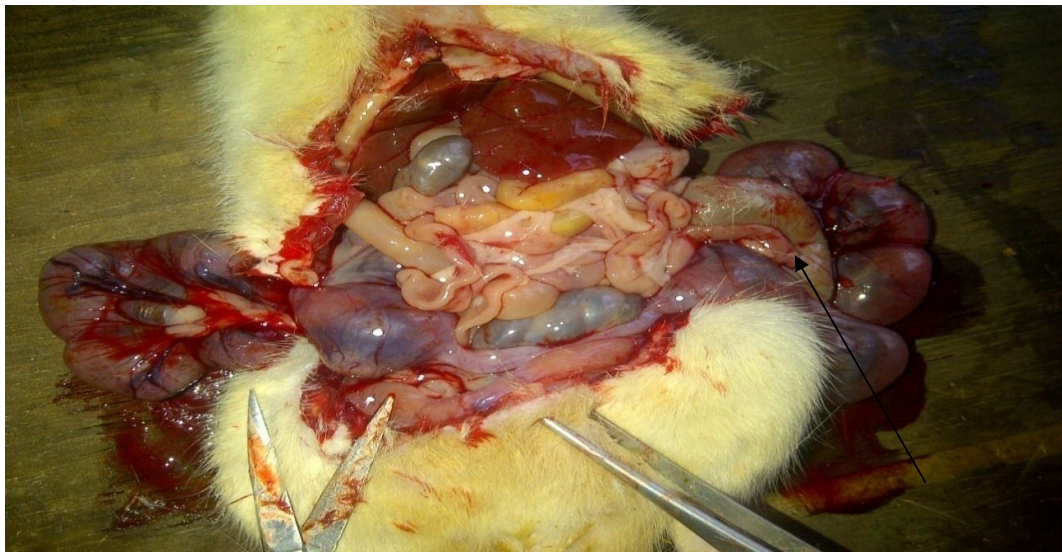


Plate 9: Arrow showing live fetuses recovered from maternal rats

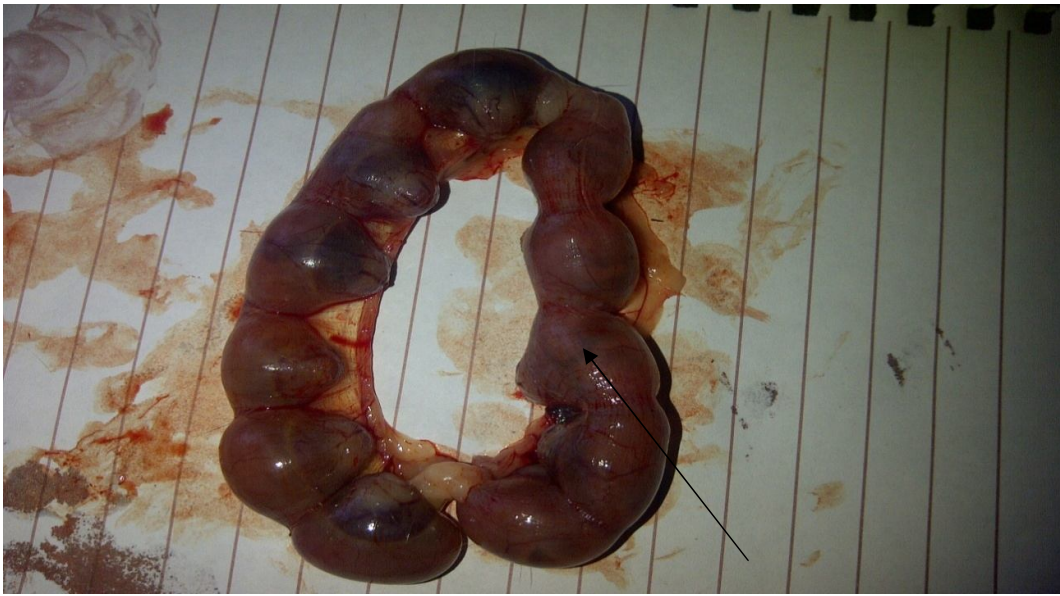


Plate10: Arrow showing live fetuses recovered from maternal rats.



Plate11: Arrow showing live fetuses recovered from maternal rats

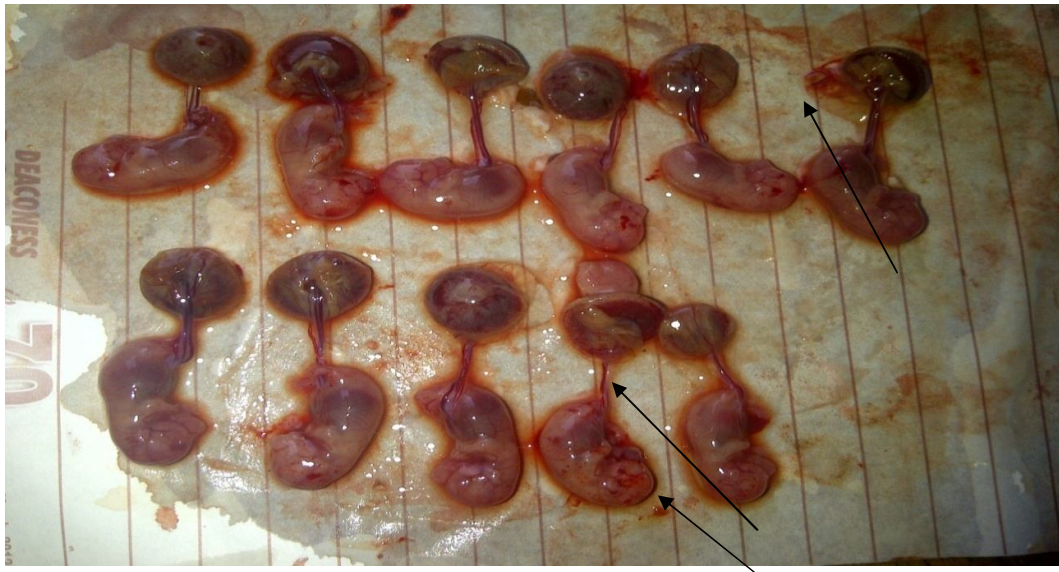


Plate12: Arrows A represent fetus, B represent placenta and C represent umbilical cord (fetal parameters) of live fetuses recovered from maternal rats after administration of extract.

DISCUSSION

Teratogens are substances that cause the occurrence of significant abnormalities either structural or functional following fetal exposure during pregnancy (Schardien and Kella, 1993). The most common effects of teratogens are spontaneous abortion, congenital malformation, intrauterine growth retardation, functional abnormalities, carcinogenesis and mutagenesis (Briggs, 1994). Exposure to some teratogens when in trace amount can have a devastating effect. Some teratogens are benign until they build up to threshold concentration level in the mother and their effect are seen.

The contraceptive effect of *Momordica foetida* (cucurbitaceae) at a high dose of 500mg/kg of body weight of extract after postcoital administration for 10 days, resulted in complete absence of fetuses. This outcome suggests an anti-implantation property of the extract which is in consistency with the report by Jonathan *et al.*, (1995), and Population Council, (2005) that some substances cause the existence of post-fertilization mechanisms including prevention of implantation and destruction of blastocysts. Perhaps the anti-implantation effects of the extract is effective enough to cause sloughing of the endometrium and leading to pre-implantation vaginal bleeding in some experimental animals. The study has revealed that the ethanolic leaf extract of *Momordica foetida* (cucurbitaceae) prevents implantation.

The obvious vaginal bleeding and lifeless fetuses found on the floor of the cages is apparently an indication of abortion. And on the sacrifice of maternal rats no fetuses were seen. Though the mechanism of action that might have led to the signs of an apparent spontaneous abortion can not be explained as it was not within the purview of this study. But it is apparently in consistency with the report that the abortifacient effect observed in group C₁ may be mediated by the estrogenic contractility effects of ethanolic leaf extract of *Momordica foetida* (cucurbitaceae) by interfering with the hypothalamic pituitary axis (Okwuasaba *et al.*, 1999; McNie *et al.*, 2003).

A significant difference existed in the feeding pattern of the 2 groups of rats (treated and control animals). Although nutritional, genetic and endocrine factors have been known to influence intrauterine growth, several environmental agents can also induce growth retardation depending on the dose and stage at which the embryo is exposed to them.

Indices used in accessing fetal growth retardation includes body weight; crown-rump length trans-umbilical cord length (Davies, 1998), Tail length and Placental weight (Hill, 1974). In the present study, the teratogenic potential of the ethanolic leaf extract of *Momordica foetida* (cucurbitaceae) have been demonstrated as most of the above named parameters were significantly reduced ($P < 0.05$) compared to the control.

Finally the anti-fertility and antigrowth effects observed in the current study could also be attributable to toxins in the leaf of *Momordicafoetida*. *Momordicafoetidaschum* (cucurbitaceae) leaf is rich in toxic proteins, the main one being triterpenoid and ribosome inactivating protein. Once inside the cells, they become deadly enzymes that catalyses the depurination of ribosome. When ribosomes loses specific purine bases such as adenine they no longer function and protein synthesis is shut down.

CONCLUSION

The current study shows that the ethanolic leaf extract of *Momordicafoetidaschum* (cucurbitaceae), when consumed at high dose (500mg/kg- wt) during pregnancy, caused spontaneous abortion and reduced fetal parameters such as fetal weight, fetal crown-rump-length, in Sprague dawley rats. Therefore pregnant women should be wary of the consumption of *Momordicafoetidaschum* (cucurbitaceae) leaf.

RECOMMENDATION

Previous works have shown that *Momordicafoetidaschum* (cucurbitaceae) have a wide range of medicinal uses which are beneficial to human health, but this recent study have also shown that *Momordicafoetidaschum* (cucurbitaceae), when consumed during pregnancy has teratogenic potential. However, further work needs to be done investigating the effect of *Momordicafoetidaschum* (cucurbitaceae) on the histology of organs of surviving fetuses to ascertain the teratogenic potential of *Momordicafoetidaschum* (cucurbitaceae) extract on the fetal organs.

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