

HAEMATOLOGICAL PROFILE, ANTICONCEPTIVE, EARLY ABORTIFACIENT AND ECBOLIC EFFECTS OF THE ETHANOL SEED EXTRACT OF *LUFFA CYLINDRICA* IN FEMALE RATS***Odo Rita Ifeoma, Uchendu Chukwuka Nwocha and Agbo Perpetual Ugochukwu**

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Article Received on 05/05/2020

Article Revised on 26/05/2020

Article Accepted on 16/06/2020

ABSTRACT

This study was carried out to determine the contraceptive, early abortifacient and ecobolic effects of the ethanol seed extract of *Luffa cylindrica* in female rats, and also the effect of the extract on haematology in female rats. Twenty four female and 8 male albino rats were used for the study. Each group containing 6 females and 2 males kept separate and used when required. Group 1 female rats confirmed to be on estrus were administered 80 mg/kg of *Luffa cylindrica* seed extract (LCSE) and paired with the males. Group 2 female rats were administered LCSE (80 mg/kg) four days post mating. Group 3 female rats were administered LCSE (80 mg/kg) on day 18 of pregnancy. Group 4 was the control, given distilled water as placebo (10 ml /kg). Female rats in all the groups were euthanized on day 20. There was no foetus in the uterus in groups 1 and 2. There were significant decreases ($p < 0.05$) in the foetal weight and foetal crown rump length of group 3 when compared to group 4, but the number of life foetuses was comparable ($p > 0.05$) between the two groups. Packed cell volume, haemoglobin concentration and red blood cells were comparable among the four groups. White blood cells and lymphocytes were markedly higher in groups 1, 2 and 3 when compared to group 4. From the results, LCSE has contraceptive, early abortifacient and immune system boosting effects but has no ecobolic effect at the dose of 80 mg/kg in female rats.

KEYWORDS: *Luffa cylindrical*, contraceptive, abortifacient, ecobolic, haematology.**INTRODUCTION**

Some medicinal plants have been used to induce labour during parturition.^[1] It has been reported that some of these plants induce abortion when used during the first trimester of pregnancy.^[1] Medicinal plants used to induce labour cause increase in uterine contractions.^[2] Plants that produce uterine contractions have similar mode of action as oxytocin which stimulates the uterus to experience strong contractions thus inducing labour.^[3] The uterus plays an important role in the female reproductive tract. This is due to the fact that it is the site of embryo implantation. Foetal development, growth, and differentiation occur in the favourable environment of the uterus, with the foetus drawing its nutrition from the rich maternal blood supply until the time when it is able to survive in the external environment. The uterus is relatively quiescent during pregnancy (when it is harbouring the developing foetus). On the other hand, at the time of labour, it enters a very active and reactive state, which is characterized by forceful, rhythmic, and synchronous contractility. This change in the uterine contractility is thought to occur due to a complex interaction between an array of systems and events.^[4] Although uterine contraction *in vivo* has been studied

widely, most studies have focused on the pregnant uterus at parturition and post partum.

The methods utilized frequently in most studies on uterine contractile activity are based on the measurement of the contractile activity of the isolated myometrium *in vitro*.^[5] Even though removal of the tissue from its natural environment and elimination of the numerous complex interactions make it easy to perform experiments and understand the underlying mechanisms, uterine smooth muscles in pregnancy undergo extensive metabolic changes to support the physiological process of labour.^[6,7]

Luffa cylindrica, also known as Sponge gourd is a fibrous plant that produces fruits with black seeds. It belongs to the family Cucurbitaceae. It is a flowering plant with pollinated flowers that develop into cylindrical green fruits. The fruit is edible especially when young and it contains group of compounds such as phenolics, flavonoids, oleanolic acid, ascorbic acid, α -tocopherol, carotenoids, chlorophylls, triterpenoids and ribosome-inactivating proteins which make it highly effective when used for medicinal purpose.^[8]

The leaf extract of *Luffa cylindrica* has been reported to induce uterine contraction and as well hastens labour in human.^[2] The seed extract has also been reported to cause contraction of isolated myometrium *in vitro*.^[9] There is paucity of information on the *in vivo* anticonceptive, early abortifacient and ecbohic effects of the seed extract of *L. cylindrica*. Hence, the aim of this study was to determine the effects of ethanol seed extract of *Luffa cylindrica* on conception, parturition and haematology in female rats.

MATERIALS AND METHODS

Animals

Twenty four female albino rats and eight male albino rats of three months old with weight ranging from 180 - 200 grams were used for the study. The animals were acclimatized for two (2) weeks before the commencement of the experiment. All animals were fed *ad libitum* on a standard commercial feed (vital feed) and clean drinking water. All protocols and handling were in accordance with the directive of Ward and Elsea and Zimmermann.^[10,11]

Plant collection and identification

Dry samples of the plant were collected from Ama-Orba Inyi in Igbo Eze North Local Government Area, Enugu state, Nigeria and was identified by Mr. A. O. Ozioko, a taxonomist of Bioresources Development and Conservation Programme, Aku Road, Nsukka.

Preparation and extraction of the *Luffa cylindrica* seed

After the collection of the dried *Luffa cylindrica* fruit, the seeds were separated from the pulp and dried under a shade. After drying the *L. cylindrica* seeds were crushed at Crop Science Department University of Nigeria, Nsukka using a crushing machine. It was defatted with hexane and then extracted with 80% ethanol. It was filtered using Wattman No 1 filter paper. The filtrate was concentrated in a hot air oven at 37°C and stored at 4°C during its use.

Experimental design

Thirty two mature rats consisting of 24 female and 8 male albino rats were used for the study. They were randomly assigned into four groups. Each group consists of six females and two males kept separately and used when required. The female rats were confirmed to be on estrus before mating.^[12] Group 1 female rats confirmed to be on estrus were administered 80 mg/kg of *Luffa cylindrica* seed extract (LCSE) and paired with the males. Group 2 female rats were administered LCSE (80 mg/kg) four days post mating. Group 3 female rats were administered LCSE (80 mg/kg) on day 18 of pregnancy. Group 4 was the control, given distilled water as placebo (10 ml /kg). The 80 mg/kg of LCSE administered to the female rats was according to previous research.^[8] Groups 1, 2 and 3 were meant to determine the anticonceptive, early abortifacient and ecbohic effects of the extract respectively. Female rats in all the groups were

euthanized on day 20, and various parameters were assessed.

Determination of number of live foetuses

On the 20th day, the female rats were euthanized, their uterus dissected out and the number of live foetuses were counted for each female rat.

Determination of foetal weight and crown rump length of the foetuses

Using a digital weighing scale, the litter weight was determined; each litter was placed on the weighing pan and allowed to stabilize and the weight in grams was taken. The crown rump length was evaluated using a thread in measuring the distance from the litter's crown down to the rump after placing each litter in a prone position. The thread was then correlated to a meter rule to determine the length measured in centimetres.

Determination of haematological parameters

Blood samples were collected from the retro bulbar plexus via the median canthus of the eyes of the rats into EDTA –contained bottles for haematology. Haematocrit technique was used to evaluate the packed cell volume.^[13] Values were expressed in percentage. Haemocytometer method was used to determine the red blood and white blood cell counts, and cyanomethaemoglobin method was used to determine the haemoglobin concentration and values recorded in g/dl.^[13] Leishmann technique was used to determine the differential leucocyte counts.^[14]

Statistical Analysis

The data obtained from the number of live foetuses, foetal weight and crown rump length were analyzed using Independent Samples T Test, and those from haematology were subjected to one way analysis of variance (ANOVA). For ANOVA, means were compared using Duncan's multiple range tests. Significant level was accepted at $p < 0.05$.

RESULTS

Anticonceptive effect

There was no foetus in the uterus of group 1 female rat on the day 20 of the pregnancy.

Early abortifacient effect

There was also no foetus in the uterus of group 2 female rats on the 20th day of pregnancy.

Number of life foetuses, foetal weight of the offsprings and crown rump length of foetuses from groups 3 and 4.

There was no significant difference ($p > 0.05$) in the number of life foetuses of group 3 (9.00 ± 2.08) when compared to group 4 (9.33 ± 1.45) as shown in Table 1. There was a significant decrease ($p < 0.05$) in the foetal weight of group 3 (4.72 ± 0.11) when compared to group 4 (5.91 ± 0.06) as shown in Table 1 and there was a significant decrease ($p < 0.05$) in foetal crown rump

length of group 3 (4.83 ± 0.12) when compared to group 4 (5.73 ± 0.15) (Table 1). There was no significant difference ($p > 0.05$) in the packed cell volume (PCV) among the four groups (1 - 48.67 ± 2.73 , 2 - 40.67 ± 2.96 , 3 - 46.67 ± 0.33 , 4 - 41.67 ± 6.94) as presented in table 2. The haemoglobin concentration and red blood cells were comparable among the groups (Table 2). There was a significant increase ($p < 0.05$) in white blood cell count of

groups 1, 2 and 3 when compared to group 4 (Table 2). Result from different leukocytic series were presented in Table 3. In the neutrophil, there was no significant difference ($p > 0.05$) among the four groups. There was a significant increase ($p < 0.05$) in the lymphocyte of groups 1, 2 and 3 when compared to group 4. There was also no significant difference ($p > 0.05$) in the eosinophil and monocytes count among the four groups.

Table 1: Mean values of the number of life foetuses, foetal weight and crown rump length of the foetuses from groups 3 and 4.

Parameters	Group 3	Group 4
Number of life foetuses	9.00 ± 2.08^a	9.33 ± 1.45^a
Foetal weight (g)	4.72 ± 0.11^a	5.91 ± 0.06^b
Crown rump length (cm)	4.83 ± 0.12^a	5.73 ± 0.15^b

Different superscript ^a and ^b indicate significant differences between the mean ($p < 0.05$)

Table 2: Mean values of the haematological parameters of the female rats

Parameters	Group 1 Extract was given before mating	Group 2 Extract was given 4 days after mating was confirmed	Group 3 Extract was given on the 18 th day after mating was confirmed	Group 4 Control No extract was given
Packed cell volume (%)	48.67 ± 2.73^a	40.67 ± 2.96^a	46.67 ± 0.33^a	41.67 ± 6.94^a
Haemoglobin Conc. (g/dl)	15.53 ± 0.44^a	15.60 ± 0.40^a	15.40 ± 0.20^a	15.40 ± 0.32^a
Red Blood Cell Count ($10^6/\mu\text{l}$)	106.67 ± 8.82^a	108.33 ± 6.01^a	110.00 ± 5.77^a	106.67 ± 8.83^a
White Blood Cell Count ($\times 10^3 \mu\text{l}$)	8.53 ± 0.70^a	7.87 ± 0.18^a	8.70 ± 0.10^a	5.73 ± 0.31^b

Different superscript ^a and ^b indicate significant differences between the mean ($p < 0.05$)

Table 3: Mean values of the different cells of the leukocytic series of the female rats.

Parameters	Group 1	Group 2	Group 3	Group 4
Neutrophil	60.67 ± 4.06^a	62.33 ± 3.18^a	67.00 ± 1.73^a	68.67 ± 1.33^a
lymphocyte	37.33 ± 3.71^a	36.67 ± 1.85^a	37.33 ± 1.20^a	29.33 ± 0.67^b
Eosinophil	2.00 ± 1.15^a	2.00 ± 1.14^a	2.00 ± 0.00^a	2.00 ± 1.15^a
Basophil	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
Monocyte	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a

Different superscript ^a and ^b indicate significant differences between the mean ($p < 0.05$)

DISCUSSION

The anticonceptive and early abortifacient effects of the ethanol seed extract of *L. cylindrica* at the dose of 80 mg/kg recorded in this study may be as a result of excessive contraction of the uterine muscles resulting in inability of the foetuses to implant in the uterus and expulsion of already attached foetuses respectively. These results of the anticonceptive and early abortifacient effects show that the extract can be used to control breeding in the cases where pregnancy is not needed. The result of early abortifacient effect agrees with a previous work that reported that oxytocic plants induce an abortion when used during the first trimester of pregnancy.^[1]

There was no significant difference ($p > 0.05$) in the number of the life foetuses of group 3 when compared to group 4. The significant decreases ($p < 0.05$) in the foetal weight and foetal crown rump length of the fetuses in

group 3 when compared to group 4 may be as a result of the effect of the extract on foetal weight. This is however in line with the study which reported that *Luffa angulata* leaf extract causes reduction in fetal weight. The significant decreases in the foetal weight and crown rump length of group 3 when compared to group 4 show that the extract can be used to reduce the sizes of foetuses prior to parturition hence can be used to prevent dystocia due to foetal oversize.

The packed cell volume, haemoglobin concentration and red blood cells are comparable in all the groups. However, this is contrary to the work which reported that the leaf extract led to a dose dependent increase in red blood cell count, haemoglobin and packed cell volume. This may be because the leaf and the seed probably do not have the same constituents, and the leaf extract was given to mice for a longer period of time.^[16]

The significant increases in the white blood cell count in groups 1, 2 and 3 when compared to group 4 ($p < 0.05$) may be as a result of the immune response of the animals to the extract. This is in line with the report that *Luffa cylindrica* brought about an increase in the total white blood cell count of African catfish exposed to the fruit extract.^[17] The significant increases in the value of lymphocytes of groups 1, 2 and 3 when compared to group 4 may be due to the ability of the extract to improve cellular immune system. The increase in lymphocytes may also be due to the presence of saponins in the extract.^[18,19] Saponins have the ability to boost immunity.^[20,21] This increase shows that the extract has the potentials of improving the cellular immune system and may be effective in management of lymphocytopenia in animals.

CONCLUSION

The result from this study showed that *Luffa cylindrica* seed extract has anticonceptive, early abortifacient and immune system boosting effects, but does not have ecobolic effect *in vivo* at the dose of 80 mg/kg. More studies are recommended to determine whether higher doses of the extract can cause ecobolic effect.

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