

Full Length Research Paper

# Contraceptive effect of *Lawsonia innermis* (henna) in the Amo women of Jengre, Bassa local government area, Plateau state using the albino rats as experimental animals

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To test if *Lawsonia innermis* have any anti-fertility effects. To test the mechanisms of action of the plant using albino rats as experimental animals and to determine if conception is present after administration of plant extract. Experimental rats were used in accordance to the WHO regulatory procedures. Wister rats weighing 35g - 45g, three males and seven females at 3 weeks of age were used. Period covered by the experiment was two months. The selection of animals for use in the study was determined by the presence of at least two consecutive 4 - day estrous cycle. The animals which were divided into three groups were administered 5mls and 10mls of extract (*Lawsonia innermis*) for four days. On the fifth day, fertile males were introduced using the 2:1 ratio (female to male) and were allowed to return with the females until the experiment was terminated. The control (third) group was given normal saline orally. The extract of the plant's root was used. Its phytochemical analysis revealed that it contained saponins, tannins, cardiac glycosides, steroids and flavonoids balsam. The median lethal dose 3200C 170mg/Kg body weight. Mating was confirmed with the presence a of dead sperm cells in the vagina smear. Following the administration of the extract, the rats were sacrificed humanely the uterus removed and sections were made, stained and viewed microscopically. The effect of this plant was found to be dose dependent causing distortion in the normal histology of the ovary, preventing the formation of follicular cavity and arresting oocytes development and maturation. It also reduced the luminal diameter of the uterus and changed its normal star shaped appearance to oval shape. The results obtained from this research indicate that *Lawsonia innermis* has anti-contraceptive properties that are dose-dependent.

**Keywords:** *Lawsonia innermis*, contraception, albino rats, Amo women.

## INTRODUCTION

### Medicinal plants - a gift of nature

Through the ages, Nature has provided humans with resources of the essentials of life, including food,

medicine and raw materials for the maintenance of clothing and shelters. In particular, higher plants have been the source of medicinal agents since earliest times, and today they continue to play a dominant role in the primary health care of about 50% of the world's population (Farnsworth et al., 1985). Natural products, and medicinal agents derived there from, are also an essential feature in the health care system of the remaining 20% of the population residing mainly in

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developed countries with more than 50% of all drugs in clinical use having a natural product origin (Baladrin et al, 1993). Of the world's 25 best selling pharmaceutical agents, 12 are natural products derived, and natural products continue to play an important role in drug recovery programmes of the pharmaceutical industry and other research organizations. Research into the chemical and biological properties natural products over the past two centuries has not only yielded drugs for the treatment of human diseases, but has provided the stimulus for the development of modern synthetic organic chemistry, and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents. Of 119 plants - derived drugs commonly in used in one or more countries, 74% were discovered as a result of chemical studies directed at the isolation of the active constituents of plant used in traditional medicine (Farnsworth et. al, 1985). Well known examples include the cardiac glycosides from *Digitalis purpurea* L., used for the treatment of cardiac failure, the anti hypertensive agents and tranquilizer, reserpine, from the East Indian Snake root, *Rauwolfia Serpentina* L., the anti malarial agent, quinine, from *cinchona* sp; the analgesics, codeine and morphine, from *papaver sonniferum* L., physostigmine, drug used for the treatment of glaucoma, extracted from calabar beans, d-tubocurare, skeletal muscle relaxant isolated from curare and emetine, an anti-diarheal active ingredient extracted from ipecacuanha. Secondary metabolites isolated from medicinal plants have also served as precursors or models for the preparation of effective agents through semi-synthesis or lead - based total synthesis. Examples include the anti cancer have been isolated from the zoapatte plant, *Montanoa tomentosa* (compositae). This plant has been used as an infusion for centuries in Mexico as an abortifacient and emmenagogue (Levine, 1980). Similarly, the efficacy of the seed of *Ricinus communis* (var. minor), often swallowed by, women in central part of Nigeria for family planning has recently been confirmed by (Okwuasaba et al 1991). Perhaps it may have been in realization of the increasing consciousness of medicinal effects of plants (Fertility regulating plants) especially as they affect the world geometric growing population that led in October, 1995, the Advisory Group of WHO's

programme of Research, Development and Research Training in Human Reproduction to recommend that a more intensive effort be directed at plant products, and that a task force be constituted (WHO, 1978).

The task force on indigenous plants for fertility regulation (anti-conceptive effects) was charged with identifying plants and plant derived materials that would be safe and effective anti-conceptive agents when used orally by humans. In every case, the initial ideas for

development of products begins with information that plants have been used by humans for some type of fertility - regulation, whether or not the effect is real remains to be established. The reason for long periods of time, there is a high degree of probability that the standardized extract or pure active principle from it will have a greater margin of safety than would products developed starting from other premises. Usually, in synthetic drug development programmes, hundreds of analogues must be synthesized and the compound found to be most active may well be too toxic for human use. It is anticipated that by starting a programme with plants known to have been used by humans for long periods, toxicity problems will be minimized (WHO 1978).

In addition to the above criteria, the WHO programme further limits its interest to the following types of agents (Farnsworth et al 1980):

- a. Those administered to women in a "morning after" basis
- b. Those administered to women "after one missed basis
- c. Just prior to expected menstruation and
- d..Those taken by men that will limit their capability to fertilize but will not affect libido.

Thus, for use in women, the programme is not interested in late stage abortifacient, embolic or anovulatory agents, but directs its efforts towards the development of agents acting prior to or shortly after implantation. Thus these drugs must be capable of preventing or disrupting implantation in women or inhibiting spermatogenesis or interfering with sperm maturation in the men (Farnsworth et al 1980).

Since the antifertility activity of many plants is known or suspected to be due to their content of phytoestrogens or other compound of known structure and biological activity (Farnsworth et al, 1976), the principal objective of the task force was to identify orally - active, non-steroidal and non - estrogenic compounds which exhibited the characteristics indicated above of the estimated 250,000 currently known higher plants species, very little is known about their active compounds and their secondary metabolites, this is particularly true for tropical flora, which constitute over 60% of this estimated number. Given the rapid destruction of tropical habitats, especially the rainforests and the degradations of some marine ecosystems, this lack of knowledge is alarming.

Considering that the 119 drugs mentioned above were isolated from only about 90 plant species, the potential for drug discovery from plants and other natural sources is enormous, but little time remains to explore this rapidly diminished source.

Although the long established traditional medicine systems such as those existing in China and India, have recorded much of their knowledge, including the use of many medicinal plants, in written texts, ethnobotanists and anthropologist have expressed alarm

at the rapid loss of the knowledge of traditional healers, particularly amongst indigenous groups in the Neotropics. Before the late 1980, the developed world displayed little interest in such indigenous knowledge, and minimal effort was expended to assist indigenous communities in preserving their unique knowledge and traditions. With the resurgence of interest in screening of plants and other natural resources for potential medicinal properties, western research organizations are beginning to place greater value on such knowledge. Where such knowledge is accessible, the research for bioactive substances might be expected to be more effective and efficient than in cases where all samples are collected with no basis for selection, this later form of collecting is often referred to as biodiversity prospecting or bioprospecting (Axt et al, 1973).

### A Need for Research in Fertility Regulating Plants

In Nigeria, a need for extensive research in plants that have anti-conceptive effects need not be over emphasized. The high rate of unemployment, fall in exchange rate of naira, ever increasing desertification/a forestation, lack of technological know-how for the establishment and maintenance of industrial and agro-based industries coupled with global economical recession in a country where population is over 100 million people highlights the need for population control (National Population Bureau, 1984). Besides, successful discoveries in this area would lead to the establishment of local pharmaceutical companies which would create job opportunities for our teeming population. Trading on both semi processed and finished herbal products will earn Nigeria much desired foreign exchange. The total global trade in herbal remedies and botanicals in 1995 totalled over \$56 billion (Iwu, 1996). Nigeria is blessed with many medicinal plants. Six of which are in demand all over the world with an estimated aggregate market of \$25 million, another twenty major plants can be cultivated here and promoted as substitutes for highly priced botanicals with a potential market of \$165 million (Iwu, 1996). If these are produced into herbal medicines and cosmetics, their net value would increase even more. This would invariably improve the availability of quality drug in the country and enhance national income (Iwu, 1996).

### Criteria for Plant Selection

The choice of the family and species of plants studied in this present work was based on popular use of the plants over a long period of time, predicted on safety,

chemical constituents, random selection and combination of criteria (Anon, 1991). It is from these perspectives, especially the former that the plant *Lawsonia Innermis* (Henna) was chosen for study.

### *Lawsonia Innermis* (Henna)

Symbol	:	LAINS
Group	:	Dicot
Family	:	Lytracode
Growth Habbit	:	Tree
Shrub		
Duration	:	Perennial
U.S. Nativity	:	Introduced

### Scientific Facts - *Lawsonia Innermis* (Henna)

*Lawsonia Innermis* (Henna) belongs to the family lythrocede. Henna is a perennial shrub native to North Africa, Asia and Australia, it is naturalized and cultivated in the tropics of America, Egypt, India and part of the Middle East. Also known as El-Henna, Egyptian Priest and mignonette tree, the species is sometimes classified as *Alba Lam*, or *lawsonia ruba*. Reading a height of up to 2 meters the plant has fragrant white or rose - red flowers.

The reported iife zone of Henna is 19 to 21 degrees centigrade with an annual precipitation of 0.2 to 4.2 meters and a sail PH of 4.3 to 8.0. Henna is planted today primarily as an ornament hedge, but its probably best known for its dried ground leaves, traditionally used to produce color fast orange, red and brown dyes.

The dried root of *Lawsonia innermis* (Henna) with an indigenous name Kujlanle and Hausa name Lalle, has an age long history in traditional medicine among the Amo Community in Bassa Local Government Area. Plateau State and its neighbourhood for family planning.

### Details of Quality Characters

Leaves contain an important cosmetic dye. The principal coloring matter is lawsone (2 hydroxy 1, - 4 naphthaquinone) which has also been used as a tropical sunscreen.

Seeds contain 10.6% moisture, 5.0% protein, 10.11% oil, 33.6% carbohydrates, 33.6% fibre and 4.8% ash.

Oil contains 1.7% behedic,, 9.61% arachidic, 15.8% stearic, 9.1% palmittic, 34.7% oleic and 29.3% linoleic acids.

Air dried leaves powder contains 9.0% moisture. 14.8% ash and 10.2% train.

## Aims and objectives

1. To test if plants have any anti-contraceptive effects
2. To test the mechanism of action of the plant using the Albino Rats as experimental animals by comparing those placed on test (plant extract) and those on standard (Normal Saline).
3. To determine if conception is presented after administration of the plant extract.

## MATERIAL AND METHODS

### Collection and identification of plants

The plant parts were collected from a farmland in Jengre, Plateau state. All the information needed about the plant were obtained from the herbalist Resident in the village. The plant was identified and authenticated by the department of Botany, University of Jos, Plateau State. The roots (*Lawsonia Innermis*) were sun dried pulverized using pestle and mortar and was stored at room temperature.

### Animals

Weaned Wistar rats weighing 35g - 45g were obtained from the University of Jos Animal House. They were ten in number, three males and seven females at 3 weeks of age. They were placed on a growers match diet to enable them grow and attain maturity. At about six weeks of age, which is when puberty is attained, the animals were separated males from females and the diet continued. The animals were housed in cross-ventilated rooms (temperature  $22 \pm 2.5^\circ\text{C}$ ). Humidity  $0.5 \pm 5\%$  and 12 hr night /12 hr day cycle) in moderate sized boxes.

After about 2 months of daily care, the animals were weighed and the females were found to be about 150g - 170g while males were 200g - 210g.

### Phytochemical analysis

The phytochemical analyses were done according to the standard methods of Trease and Evans (1989); Odebiyi and Sofowora (1978) using hot water extraction.

## Contraceptive activity

### Determination of Contraceptive Effect of Extract on Adult Female Rats

For this study, adult female rats, having a regular estrous cycle confirmed by daily vaginal smear Analysis were used (Okwuasaba et al 1991). The selection of animals for use in the study was determined by the presence of at least two consecutive 4 - day estrous cycle. The animals which were divided into three groups were administered 5mls and 10mls of extract (*Lawsonia Innermis*) for four days. On the fifth day, fertile males were introduced using the 2.1 ratio (female to male) and were allowed to return with the females until the experiment was terminated. The control (third) group was given orally normal saline. Mating was confirmed with the presence of dead sperm cells in the vagina smear.

### Tissue preparation

Adult non pregnant female albino rats placed on plant extract were killed by the head blow method. The abdomen was opened the uterine horns severed at the junctions with the fallopian tubes cleared of adhering mesentery and placed in ascending series of alcohol from 70% alcohol to Absolute Alcohol. After which sections were made, stained and viewed microscopically.

## RESULTS

### Contraceptive activity

#### Contraceptive effect of the extract on adult female rats

The contraceptive effect of *Lawsonia innermis* extract in the female wistar rats is as shown in table 1. The extract protected the rats from conception for 1 gestational period which was the time the experiment was terminated.

### Phytochemical analysis

The phytochemical analysis of the extract showed that the tannins saponins, cardiac glycosides, terpenes and

**Table 1.** Control group showing the measurement of lumen diameter and muscle thickness (in mm and  $\mu\text{m}$ ) with standard deviation

Slides								
1	27	11.1	-1.22	1.5	2.0	15.0	-0.3	0.09
2	26	11.5	-0.82	0.5	20	15.0	-0.3	0.09
3	27	11.1	-1.22	1.5	19	15.8	0.5	0.25
4	27	11.1	-1.22	1.5	21	14.3	-1.0	1.0
5	19	15.8	3.48	12.1	20.1	15.0	-0.3	0.09
6	20	15.0	2.68	7.2	19	15.8	0.5	0.25
7	26	11.5	-0.82	0.7	19	15.8	0.5	0.25
8	26	11.5	-0.82	0.7	19	15.8	0.5	0.25
		$\Sigma = 98.6$		$\Sigma (x - \bar{x})^2 = 25.9$		$\Sigma x = 122.5$		$\Sigma (x - \bar{x})^2 = 2.27$
		$\bar{X} = 12.32$				$\bar{X} = 15.3$		

Where  $\Sigma x$  = Summation of values in  $\infty\text{m}$   
 $\bar{X}$  = Average of values in  $\infty\text{m}$

**Table 2.** 5mls of extract showing measurements of lumen diameter and thickness of muscle (in mm and  $\mu\text{m}$ ) with standard deviation

Slides								
1	15	20.0	1.3	0.7	16	18.8	0.5	0.25
2	16	18.8	0.1	0.01	17	17.7	-0.6	0.36
3	18	16.7	-2.0	4.0	17	17.7	-0.6	0.36
4	12	25.0	6.3	39.2	16	18.8	0.5	0.25
5	12	25.0	6.3	39.7	16	18.8	0.5	0.25
6	18	16.7	-2.0	4.0	17	17.7	-0.6	0.36
		$\Sigma = 112.2$		$\Sigma (x - \bar{X})^2 = 89.11$		$\Sigma x = 109.5$		$\Sigma (x - \bar{X})^2 = 1.83$
		$\bar{X} = 18.7$				$\bar{X} = 18.3$		

steroids, and Balsam while Alkaloid and carbohydrates were absent.

### Acute toxicity

The median lethal dose of the extract was calculated using the probit values. The median lethal dose of *Lawsonia Inermis* was 3200mg/kg body weight. However, the extract showed physical signs of toxicity which ranged from decreased motor activity, loss of appetite, increased respiratory rate which was followed by restlessness and gasping to death.

## Effect of Extract on Histological Tissues

### A. Ovary (Control)

The surface of the ovary is covered with germinated epithelial cells and it shows ovarian follicular cells in various stages of development each containing a developing ovum (slide 1).

### B. Ovary 5mls Extract

The development ovaries follicle does not develop any

**Table 3.** Test 2 10mls of extract showing measurements of lumen diameter and muscle thickness (in mm and  $\infty$ m) with standard deviation.

Slides	Lumen	Thickness	Thickness	Thickness	Lumen	Thickness	Thickness	Thickness
1	14	21.4	0.6	0.36	11	27.3	3.2	10.2
2	14	21.4	0.6	0.36	13	23.1	-1.0	1.0
3	14	21.4	0.6	0.36	16	18.8	-5.3	28.1
4.	16	18.8	-2.0	4.0	11	27.3	3.2	10.2
		$\Sigma = 83.0$		$\Sigma (x-X)^2 = 5.1$		$\Sigma x=96.5$		$\Sigma(x-X)^2$
		$X = 20.8$				$X=241$		$=49.5$

Standard Deviation =  $\sqrt{\frac{\Sigma (x-X)^2}{n}}$  .....(8)

Where n = Number of slides

$\therefore$  Lumen diameter control S.D =  $\sqrt{\frac{\Sigma (x-X)^2}{n}}$

=  $\sqrt{\frac{5.1}{4}} = 3.7$

**Table 4.** Standard deviation values for lumen diameter and muscle thickness from control group to test groups (I and ii)

	Control group		Text I:5mls Extract		Test II:10mls Extract	
	Lumen diameter	Thickness of muscle	Lumen diameter	Thickness muscle	Lumen diameter	Thickness muscle
Deviation (SD)	3.7	0.7	3.9	0.6	1.3	4.1

follicular cavity. This shows an arrest in the oocyte development and maturation. (Slide 2).

### C. Ovary 10mls Extract

Cell tumours arises in the ovarian follicular epithelium. This tumour consist of closely packed polyhedral granules cells as shown in slide 3.

### D. Uterus Control

The endometrium consist of epithelial lining that rest on numerous uterine glands present in the stroma. The myometrium mainly consist of bundles of smooth muscles fibres among which are blood vessels and connective tissue fibres. It shows the inner circular and the outer longitudinal muscles. The lumen presents a star shaped structure. The measurements of the muscle thickness and lumen diameter were obtained using the graticule and this is shown in table 4 and slide 1.

### E. Uterus 5mls Extract

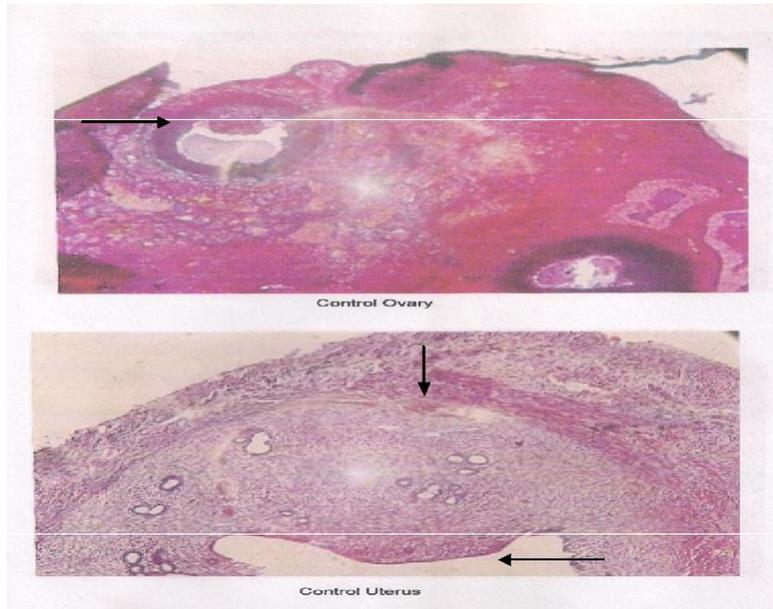
The uterine lumen presents an oval shaped structure. Present also in the stroma are numerous uterine glands. The Myometrium consist of bundles of smooth muscles fibres among which are the blood vessels and connective tissues fibres. The shows the inner circular and the outer longitudinal muscles. The measurements of the lumen diameter and muscle thickness were done using the graticule. This is shown in slide 2.

### F. Uterus 10mls Extract

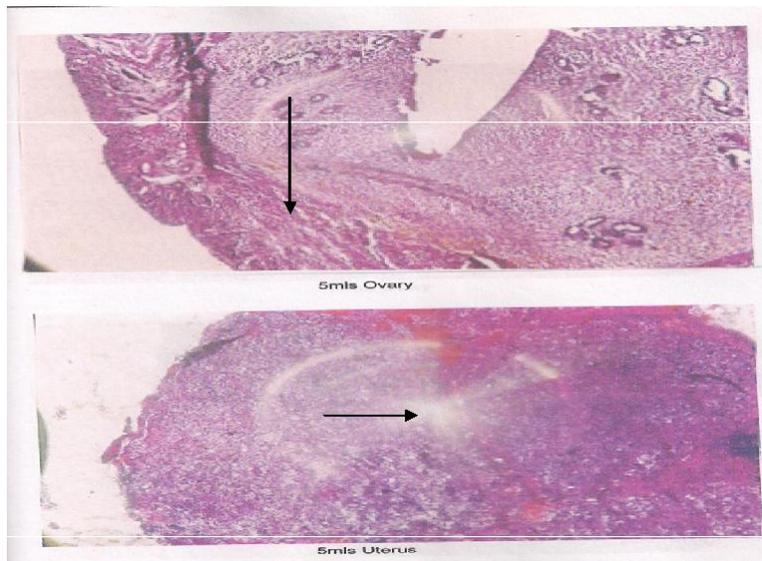
The uterine lumen presents an oral shaped structure. Found in the stroma are few uterine glands. The myometrium consist of bundles of smooth muscles fibres among which are the connective fibres and blood vessels. It shows also the inner circular and outer longitudinal muscles (Slide 3)

The measurements of lumen diameter and muscles thickness was obtained using the graticule and the

Histological slides



**Slide 1.** Above; Arrow right shows a normal follicle developing.  
Below; arrow left shows the uterine lumen; arrow down shows the myometrium



**Slide 2.** Above- Arrow down shows the myometrium.  
Below- Arrow right shows a narrow oval-shaped uterine lumen

values tabulated in table 4.

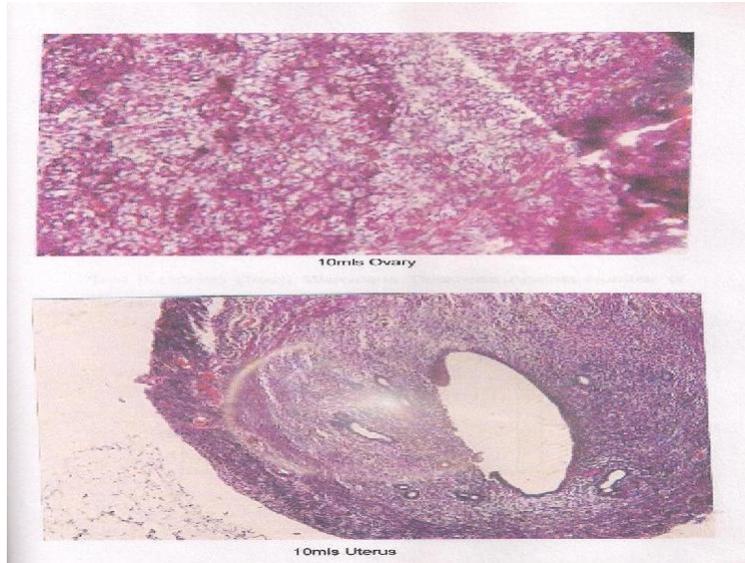
The data was obtained using the micrometer graticule and was adjusted using the eyepiece graticule to determine the calibrating factor.

Eye piece graticule corresponds at 90 and 35 while stage graticule corresponds at 21 and 11

$$\text{Calibrating Factor} = \frac{21-11}{70-35} = \frac{10}{35} = 0.28$$

∴ Calibrating Factor = 0.3 ≈

$$\text{Calibrating factor} = \frac{\text{Stage Graticule}}{\text{Eye piece graticule}}$$



Slide 3. Above- Follicular development arrested.

Graphs

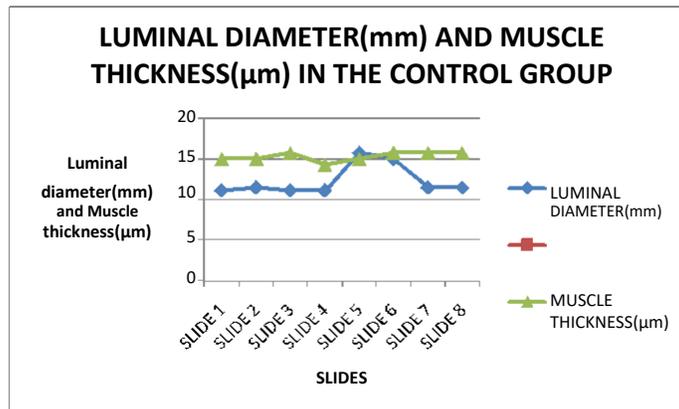


Figure a

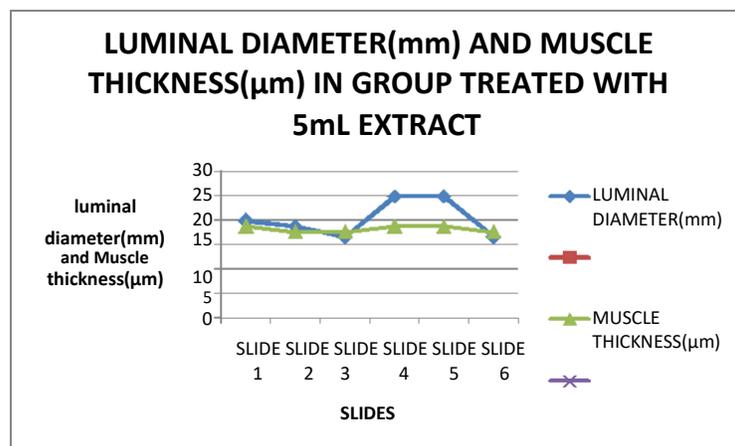


Figure b

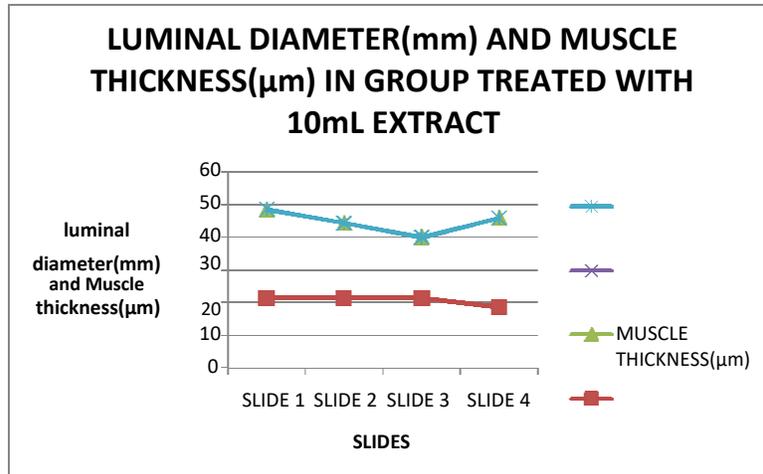


Figure c

## DISCUSSION AND CONCLUSION

### Antifertility

#### The Contraceptive Effect of the Extract *Lawsonia Innermis*

The present findings indicate that the extract of *Lawsonia Innermis* root possess, anti-conceptive activities in the white Albino Rats. This conclusion is supported by the following observations.

1. The pretreated females that were left with fertile males were protected from conception for one gestational period.

2. Signs of conception were not noticed in pretreated animals at midpoint of gestational period (21 days) as compared with those on control group. Although the mechanism of action of lawsonia innermis extract cannot be delineated from this study, it may be connected with the following:
  1. It may kill spermatozoa or ova in the female genital tract.
  2. if the zygote is formed, it may be blastocidal and the dead blastocyst cannot implant in the uterus.
  3. Even if the blastocyst is formed, it may not be welcomed by the unprepared uterus since it may denature the endometrial lining of the uterus.

### Phytochemical analysis

Some effect of the extract is reflections of their chemical constituents. The phytochemical analysis of the extract revealed that they contained flavonoids, tannins, saponins, cardiac glycosides terpenes and steroids and Balsam.

Saponins, tannins and flavinoids are known ulcer productive agents (Aguwa and Nwako, 1988, John and Onabanjo, 1990). Besides saponin-rich and phytoestrogen (steroids) constituents are implicated in their anti-conceptive property. (Farnsworth et al 1988). Saponin also has haemolytic effect. Flavonoids have anti-inflammatory, anti-allergic, anti-throbotic, vasoprotective properties, inhabitation of tumour promotion, antibacterial, and antifungal effects.

(Trease and Evans, 1989). Indeed the presence of flavonoids and other active constituents in these extract could be potential sources of useful pharmaceutical products.

Cardic glycosides are compounds with complex nature in plants. They contain special sugar constituents and aglycones that have the property of stimulating the heart muscles (Godwin and Mercer, 1983). These are referred to as cardiac active or cardiotoxic glycosides (Godwin and Mercer, 1983). Besides the cardiac effects, the cardiac glycosides can also produce gastroenteritis and diarrhea, many of the other signs are a direct result of the inability of the heart to circulate blood. Signs of poisoning usually develops 4 – 12 hours after ingestion of the plant and, if lethal quantities are taken, death occurs in 12 – 24 hours, with sub lethal quantities the clinical signs may persist for 2 – 3 days.

### Acute toxicity

The physical signs of toxicity which ranged from decrease in motor activity, increase in respiratory rate which was followed by restlessness, gasping to death might explain both the peripheral and the central effects of the extract.

The extract could be described as being slightly toxic.

However, since a knowledge of pharmaceuticals of a drug apart from the dose is also important in determining the degree of toxicity, toxicological studies of these plants are far from being complete.

## Effect of extract on histological tissues

### Ovary

In the normal histology of the ovary, the ovary is covered with germinal epithelial cells showing the ovarian follicular cells in various stages of development each containing a developing ovum and this was observed microscopically in the sections of the ovaries of animals placed as control (slide 1). In the pretreated animals these features were disrupted and the effect was dose dependent. In the animals treated with 5mls of the extract, the developing ovarian follicle does not develop any follicular cavity and this implies that there is an arrest in the oocyte development and maturation (slide 2).

In pretreated animals with 10mls of the extract, tumour-like structures arise in the ovarian follicular epithelium. This tumour-like structure consists of closely packed polyhedral granulosa cells (slide 3).

### Uterus

The normal histology of the uterus presents the endothelium and myometrium. The endothelium consists of the lamina propria, epithelial lignin, glands and the uterine arteries while the myometrium consists of the smooth muscle fibres, uterine arteries and interstitial connective tissue fibres. The muscle arrangement presents the inner circular and outer longitudinal muscle arrangement and lumen diameter is star shaped.

Comparing the normal histological presentation with research presentations shows that the animals placed on the control group show the same histological features with the normal while the effect of the extract *Lawsonia Innermis* which was dose dependent showed variations in the histological presentation. In pretreated animals with 5mls of the extract, the lumen diameter became narrow and oval shaped and it consists of few glands while the muscular arrangements remained the same with control group. The same observations were noticed in animals pretreated with 10mls of the extract and the uterine glands were fewer in number.

## SUMMARY OF RESULTS

The present study indicates that:-

1. *Lawsonia Innermis* extract possesses contraceptive effects.
2. Phytochemical Analysis of *Lawsonia Innermis* extract revealed the presence of saponins, tannins, flavinoids, cardiac glycosides, terpenes and steroids

and Balsam.

3. Acute toxicity study showed that the median lethal dose of *Lawsonia Innermis* extract was 3200, 150mg/kg body weight.

4. On invitro studies, *Lawsonia Innermis* mediates its effects by distorting the normal histology of the ovary and preventing the formation of the follicular cavity and arresting oocyte development and maturation. It also reduced the lumen diameter of the uterus and changed its normal histological from star shaped to an oval shaped structure. The effect of the extract was observed to be dose dependent.

## CONCLUSION

The results obtained from this research agree with the claim by some herbalists that *Lawsonia Innermis* (Lalle) has antifertility properties. And some of the effect could not be fully investigated and understood within the scope and limitations of the work.

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