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THE EFFECTS OF LAWSONIA INERMIS SEEDS ON ESTRUS CYCLE AND REPRODUCTIVE HORMONES OF FEMALE ALBINO RATS

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ABSTRACT

Lawsonia inermis (henna) family Lythraceae is used in many parts of the world as colorant and dye, beside other therapeutic uses. This study was conducted to determine the effect of the seeds extract on uterine contraction, estrous cycle, serum levels of reproductive hormones, and subsequent effects on ovaries and uterus histology. Method: Eighteen regularly cycling female albino rats were selected and divided into three groups of six each; one control receiving normal saline orally 10ml/kg once daily. The second and third groups received the ethanolic extract of the seeds at doses of 0.5 g/kg and 1.0 g/kg orally once daily for two weeks. Animals were examined two weeks after stopping treatment to see if the effects were reversed. Another group of 18 animals divided similarly, were given the extract and normal saline for two weeks. Twenty four hours after the last dose blood samples were collected for hormonal assay. Animals were sacrificed under light ether anesthesia; uteri and ovaries were quickly removed and weighed, then preserved in formal saline to prepare histological slides later. Results: The extract caused significant uterine contraction. The estrous cycle was extended to 5.45 ± 0.24, and 5.68 ± 0.13 days in the 0.5 and 1.0 g/Kg groups respectively compared to 4.2 ± 0.2 days in the control group. All estrous stages were extended significantly in the treated groups. The cycles returned to normal after stopping the doses. Serum levels of estradiole and progesterone were lower in treated groups than control; however, the reduction was not statistically significant. The weight of the ovaries was reduced significantly ($p = 0.01, 0.026$ respectively) in the treated groups, the uteri weight were also reduced yet statistically insignificant.

Histopathological study showed changes in ovarian and uterine tissues. Conclusion: The ethanol seeds extract of L. inermis is uterotonic, disrupts the estrous cycle reversibly, reduced estrogen and progesterone levels and reduced ovarian and uterine weights.

KEY WORDS: Antifertility, uterotonic, L. inermis, ovaries, progesterone.

INTRODUCTION

Lawsonia inermis (henna) family Lythraceae is a tall shrub or small tree about 2.6 m hight. Henna flowers are fragrant white or red, fruits are small, brownish capsules filled with seeds.[1] Henna leaves are famous for the lawsone dye they produce, which is used in coloring and decorating hands, feet, skin and hair.[2,3] It is native to tropical and subtropical regions of Africa, Southern Asia, and Northern Australasia in the semi-arid zones in latitudes between 15° and 25° North and South. It is cultivated commercially in many parts of the world.

Lawsone (2-hydroxy-1,4 naphthaquinone) is the chief constituent, it is responsible for the dying properties of the plant. The dried leaves contain about 0.5-1.5% lawsone.[4] Henna contains phenolic glycosides[5], alkaloids, anthocyanins, phenols, sterols, xanthoproteins[6,7,8,9] The structure and content percent of a number of constituents have been identified; examples include lawsoniaside, llalioside, luteolin, glucopyranoside and trihydroxynaphthalene, apigenin, luteolin, 2-methoxy-3-methyl-1,4 naphthoquinone.[10,11,12,13,14]

L. inermis has many therapeutic effects that were well documented these include, analgesic and anti-inflammatory action[15], hypoglcaemic[16,17,18], antibacterial[6,19,20,21,22,23], antioxidant[10,24,25], hepatoprotective[26, 27], cytotoxic effects[28,29,30], nootropic[31,32], wounds and ulcers healing beside benefit in eczema and psoriasis[33,34,35,36] are known.

A dose-dependent abortifacient activity of methanolic root extract was[37] attributed to toxicity on maternal and foetal tissues. However, no antifertility effect was observed with seed extracts.[38]

Henna is used traditionally for a number of uses and is widely accepted by lay people in developing regions, its use as contraceptive that is natural, effective and safe which could be an alternative to hormonal contraception.

Objectives: The study aims to determine the effects of L. inermis seed extract on isolated uterus, estrous cycle, estrogen progesterone levels, the weight and histology of ovaries and uteri.

MATERIALS AND METHOD

1. Materials

1.1. Plant collection

Fruits were collected from domestically grown trees near the residence of the investigator in Omdurman City, Sudan. The ripe green or dry fruits were collected and allowed to dry in the shade. The fruits were recognized and identified by a taxonomist, Department of Botany, Faculty of Science; University of Khartoum. A sample was kept for record.

1.2 Animals

Female Swiss albino rats weighing 130- 170 g were used for this study. The animals were bred and housed in the Faculty of Pharmacy, University of Khartoum animal house. They were kept under international standards of animal keep and breed. The animals were given a balanced feed, allowed free access to food and water, kept in a light/darkness cycle of 12 hrs approximately.

1.3 Extraction

The dry fruits were crushed to reduce their size. 40g of the powder was mixed with 100ml 70% ethanol and left to macerate over 24 hours under mild shaking. The material was then expressed and allowed to dry at room temperature; a solid dark brown material with a fragrant smell was produced. The extraction yield was 11.6%. Doses were prepared later after weighing and dissolving in distilled water.

2. METHODS

2.1. The isolated uterus

The uterus was extracted from a freshly killed animal and suspended in De Jalon's solution at 32^{o}C. The tissue was allowed to adapt, isotonic contractions were recorded. Acetylcholine was used as standard. Different doses of the extract were then used, and responses recorded. Each dose was repeated three times, the means were used to plot dose response curve and determination of ED_{50}.

2.2. The estrous cycle

The study adopted the vaginal smear method to identify the four stages of the female rat estrous cycle. The unstained smear method was employed.[39] The smears were prepared by lavage with normal saline. A small volume of normal saline was inserted into the vagina using a plastic pipette. A drop of the washed saline was placed on a slide and examined under light microscope (x10, x40) to identify three main types of cells; epithelial, cornified and leukocytes and accordingly determine the stage of esotrous. Rats that showed regular proestrous, eostrous, metestrous (dieostrous 1) and dieostrous (dieostrous 2) for three consecutive cycles were considered regular and included in the experiment.

Eighteen adult female rats, with regular eostrous cycle were selected and randomly allocated into three groups; the control group received normal saline orally by gavage , the other two groups received L. inermis 0.5 g/Kg and 1.0 g/kg40 mg/Kg. Doses were given for two weeks and the animals were observed for estrous stages. After two weeks from the last dose estrous cycle was observed in the treated animals to find whether the effect is reversed or not.

2.3. Hormonal assay

Eighteen female rats with regular cycle were randomly divided into three groups similar to the above experiments. The doses were given orally for 15 days. Twenty four hours after the last dose, blood samples were collected onto a plain blood container and allowed to clot. The clotted blood was centrifuged to obtain sera; which was kept frozen until analysis. Levels of estrogen, progesterone, LH and FSH were determined and recorded.

2.4. Reproductive organs weight

The animals from the above experiment were sacrificed; ovaries and uteri were rapidly removed from the animals and weight to the nearest decimal in a sensitive balance.

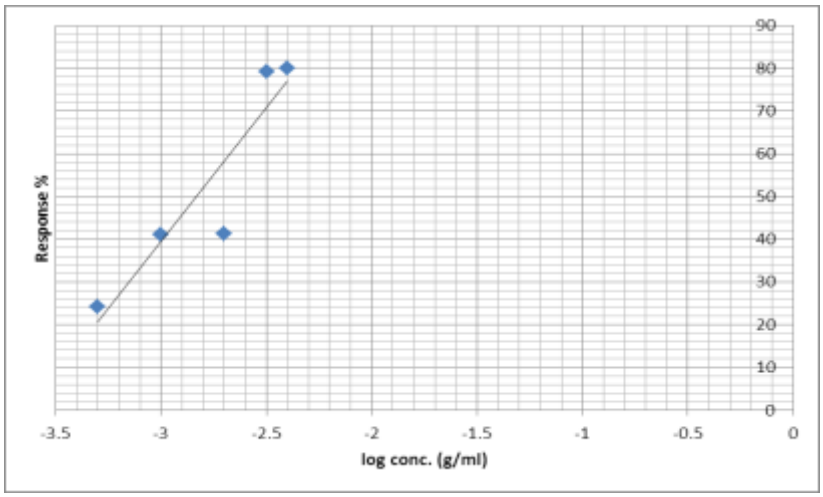
2.5. Histopathology

Ovaries and uteri were kept in formal saline, then cut and stained in haematoxylene and eosin. The slides were studied by a pathologist.

2.6. Analysis

The results obtained were statistically analyzed using Computer soft ware Excel Microsoft © and Statistical Package for Social Sciences SPSS 17 ©. The student (t) test and ANOVA were used to test the significance.

RESULTS



ED50= 1.4x10^{-3} g/ml

Figure 1: the effect of L. inermis on non-pregnant rat uterus

L. inermis produced strong contraction on rat uterus, increased frequency and magnitude of contractions. The effect produced was terminated when the extract washed out indicating the reversible nature of extract-tissue interaction. About 80% of the maximum response produced by acetylcholine was produced by the maximal dose of *L. inermis* extract. ED50 was 1.4 x 10^{-3} g/ml the dose response curve indicated efficacy.

Table 1: The effect of L. inermis on the length of the different cycle stages

Group			Stage length days ± SEM			
		Cycle length Days ± SEM	Proestrus	Estrus	Metestrus	Diestrus
A	4.2 ± 0.2	4.2 ± 0.2	0.80 ± 0.4	1.4 ± 0.6	0.80 ± 0.02	1.10 ± 0.08
B	5.45 ± 0.24	5.45 ± 0.2	0.98 ± 0.05	1.7±0.19*	1.18±0.06*	1.52 ±0.23*
C	5.68 ± 0.13	5.68 ± 0.13	0.998 ± 0.21*	1.76± 0.24*	1.58±0.29*	1.38±0.33*

*n=6. *statistically significant*

Proestrus: group C p = 0.037

Estrus: group B: p = 0.006, group C p = 0.009

Metestrus: group B 0.009, group C 0.002

Diestrus: group B 0.052, group C 0.003

Table 1. showed thatthe estrus cycle in treated rats was extended to 5.45 ±0.24 and 5.68 ± 0.13 days in the 0.5g/kg and 1.0g /kg respectively, compared to 4.2 ± 0.2 days in the control group. The sequence of the different phases was preserved but all stages took longer compared to untreated animals. Estrus (1.7±0.19 in 0.5g/kg and 1.76± 0.24 in 1 g/kg group)

was significantly longer (p <.006, p<.009), metesrus and diestrus showed similar results to estrus. Proestrus was slightly extended significantly (p< 0.037) in the 1.0g/kg group. The proestrus slides showed predominance of nucleated epithelial cells in four rats out of six in the different doses of L. inermis treated groups. Estrus cycle returned to normal after cessation of treatment for 2 weeks.

Table 2: Effects of L. inermis on ovarian and uterine weights

Group		Body weight Day zero Mean ±SEM gr.	Body weight Day 15 Mean ± SEM gr.	Ovaries wt. Mean ± SEM gr.	Uterus wt. Mean ±SEM gr.
A	**Control**	135.75 ± 4.35	150.5 ±2.25	.11 ± .008	.284±.085
B	**L. inermis 0.5g/Kg**	129.25 ±7.23	137.0 ±8.84	.064 ±.009*	.146±0.039
C	**L. inermis 1.0g/Kg**	146.5± 4.72	151.25 ± 1.31	.077 ±.008*	.166± 0.025

*N= 6 * Gr. B p= 0.01, Gr. C p= 0.026*

There is a slight increase in weight in the control group (table 2), however, the groups taking L. inermis showed minor changes in weight. The extracts produced a reduction in the uterus weight, but were not statistically significant. The reduction in ovarian weight in all treated groups was statistically significant (p = 0.01, 0.026 respectively).

Table 3: The effect of L. inermis on reproductive hormones

Group	Estradiol Mean ± SEM ng/ml	Progesterone Mean ± SEM ng/ml
Control	139.3 ± 21.0	18.76 ± 6.9
L. inermis 0.5g/Kg	124.05 ±14.11	8.32 ± 2.59
L. inermis 1.0g/Kg	137.8± 2.15	8.93 ± 2.85

n = 6

Estradiol levels in the group taking 0.5g/Kg showed greater reduction (124 ng/dL) than the 1.0 g/Kg group (137.8 ng/dL) compared to 139 ng/dL for control, yet the reduction was not statistically significant. Progesterone levels demonstrated greater reduction in both treated groups but were also insignificant statistically; 8.32 and 8.93 respectively compared to 18.76 ng/dL in control group. The ratio of estrogen: progesterone was imbalanced in the treated groups. Serum levels of LH and FSH were below 2 ng/dL in all groups. (table 3.)

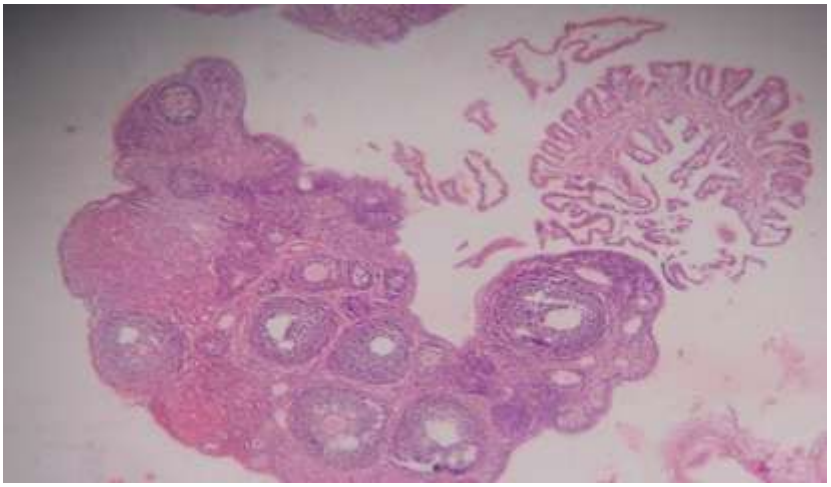


Figure 2a: Normal ovariy H7 E x40

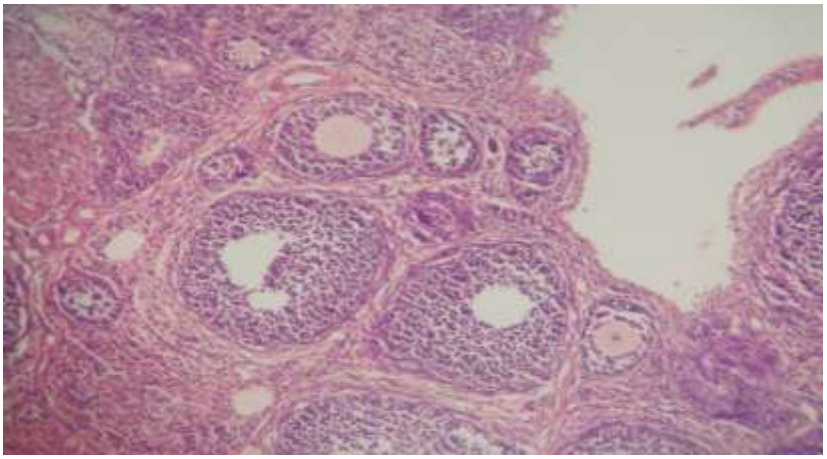


Figure 2b: Normal ovary H &E x 100

Figure 2: a & b show ovaries from control females, with follicles at various staged of development and dense stroma.

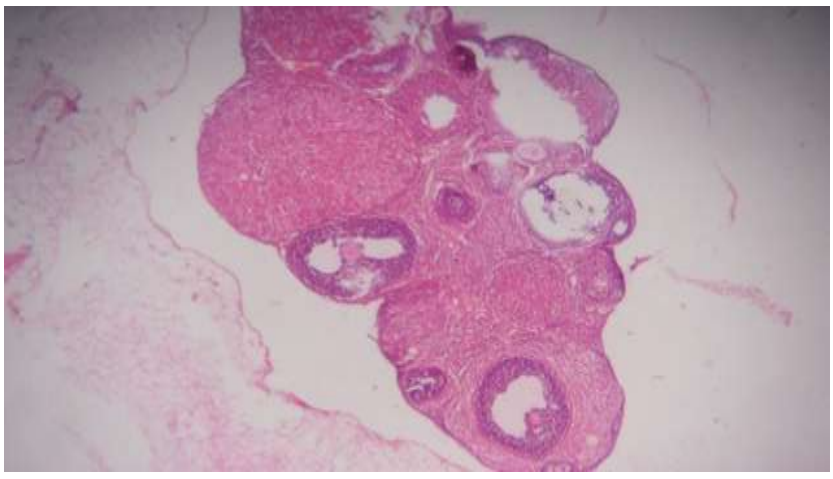


Figure 3a: ovary from rat treated with L. inermis. H&E x 40

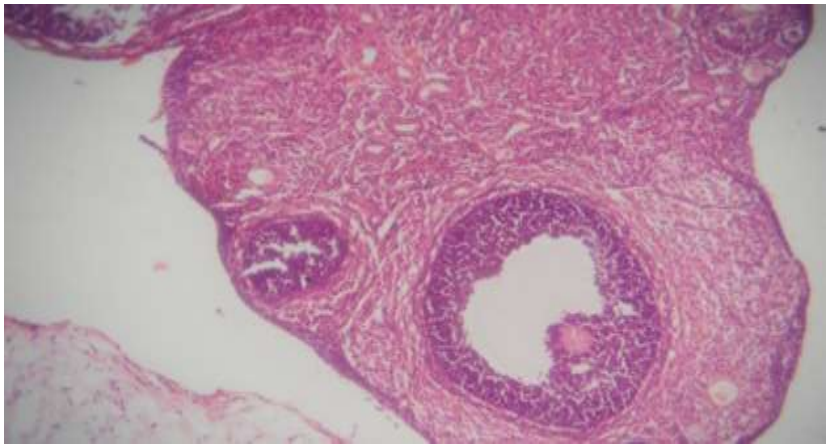


Figure 3b: ovary from rat treated with L. inermis.H&E x100

Figure 3a & B: ovaries from female rats treated with L. inermis show multiple cystic follicles, not well developed. Magnification showsartetic follicles. The stroma is loose.

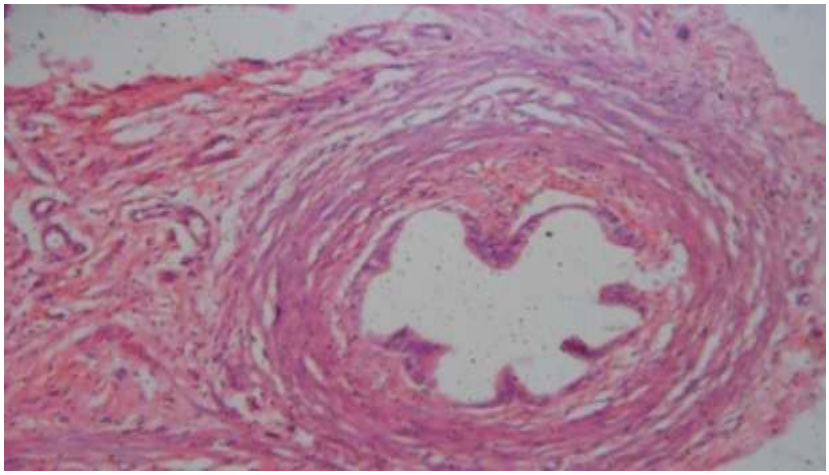


Figure 4 a: H&E x 40

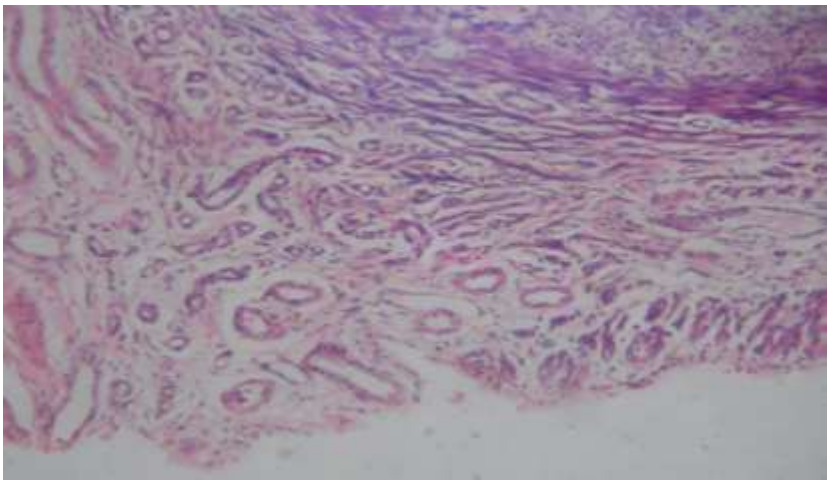


Figure 4 b: H&E x100

Figure 4 a& b: uterus from the control group showing normal, numerous glands, and dense stroma.

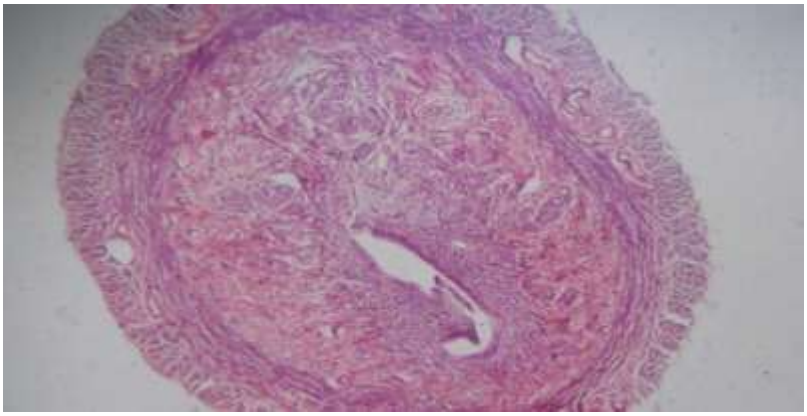


Figure 5a: H&Ex40

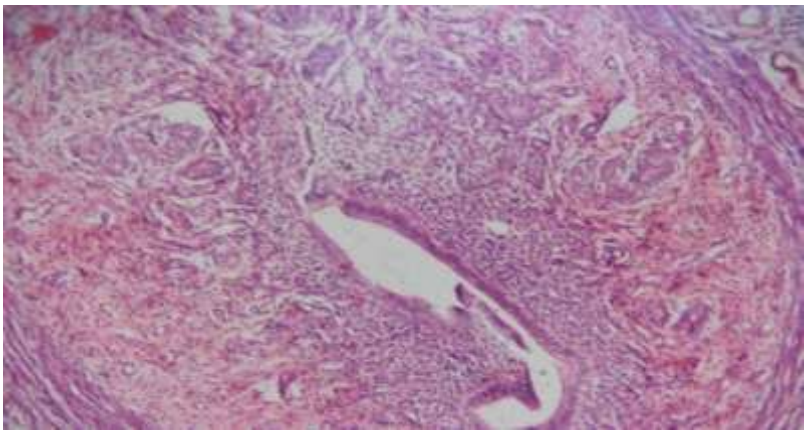


Figure 5b: H&E x 100

Figure 5 a&b: uterus from female rats treated with L. inermis

Figure 5 a & b show uterus with reduced lumen, degenerated stroma, and fewer glands. The epithelial lining is reduced.

DISCUSSION

Pretreatment of the animals with estradiol before extraction of the uterus increases the sensitivity of the uterine muscles to oxytocin, acetylcholine, prostaglandins[40,41,42] and other agents that excite uterine tissues. In this study similar results were obtained from rats exposed to estrogen, and unexposed to estrogen, the resultant contraction produced by L. inermis extract was comparable to acetylcholine. This states that the extract is efficous and its action does not depend on estrogen pretreatment or sensitization.

Uterotonic activity might indicate that the extract have abortifacient activity; this is in accordance with the traditional use as abortifacient. The underlying mechanism of many abortifacient plants is uterotonic activity, an example is *Rumex steudelii* [43]

The estrus cycle is under control of pituitary hormones LH and FSH and local hormones progesterone and estrogen.[44] Disturbances in any of these hormones would affect the normal cycle. Increment in cycle length indicates antifertility effects. The significant extension in proestrus and estrus stages denotes steroidal content of the extract. *R. sublobata* produces effects similar to those observed by the plants in this study. This medicinal plant increase estrus, proestrus metstrus and diestrus in treated female rats.[45] The authors proposed that estrogens as indicated in the steroidal and sapponins constituents of the medicinal plant are responsible for effects on estrus cycle. Malashetty and Patil[46] studies on *Crotalaria Juncea* seeds documented increased estrus and metestrus stages while proestrus and diestrus were reduced. This was attributed to estrogenic effect of the plant, that caused reduced gondotrophins (LH, FSH) through feedback mechanism.

Reduction on hormonal levels was recorded in the groups treated with *L. inermis* ; progesterone greater than estrogen. However, this was not statistically insignificant but was enough to cause disruption of estrous cycle and reduce ovaries and uteri weights.

Aspilia Africana, a medicinal plant rich in saponins, saponin glycosides, steroids, tannins, volatile oils and alkaloids; disrupt estrus cycle, and reduce the duration of proestrus, estrus and metestrus while increasing diestrus[47] due to the high phytoestrogen contents. Despite the estrogenic activity of this plant; uterus weight is reduced, the authors justified this by the ability of the plant to contract smooth muscles thus bringing about atrophy. According to the above findings, henna possess similar constituents.

Anti-gonadotrophic effects can increase the duration of estrus cycle. For example *Nelumbo nucifera* has potent effect evidenced by reducing ovarian and uterine weights, caused increased estrus cycle duration with marked extension in diestrus phase.[48] Proestrus and metestrus phases of the cycle were reduced. Sharma et al.,[49] documented antigonadotrophic effects of *Piper betle* that produced marked reduction in reproductive organs weight and prolonging estrus duration. The treated rats became anestrus with extended metestrus and diestrus phases. These effects were attributed to decreased levels of circulating estrogens due to inhibition of the pituitary-gonadal axis. *Momordica cymbalaria* reduces the duration of cycle through influencing estrogen levels.[50] Proestrus phase is increased which indicate non-maturation of follicles probably due to lack of gonadotrophin. The plant also reduced the weight of reproductive organs.

CONCLUSION

L. inermis caused significant uterine contraction comparable to acetylcholine, elicited elongation of the estrus cycle, significantly extending all stages of the cycle. Estrogen and progesterone levels were reduced insignificantly, yet ovaries and uterine weights were reduced demonstrating gonadotrophic suppression. The studied plant might therefore possess antiovulatory, atnimplantation and abortifacient activities, and might prevent fertilization due to increased motility.

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