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## **EFFECT OF ETHANOLIC ROOT EXTRACT OF HENNA (*LAWSONIA INERMIS*) ON THE HISTOLOGY OF THE OVARY OF ADULT FEMALE WISTAR RATS**

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## **ABSTRACT**

### **Aim**

The use of *Lawsonia inermis* as a medicinal and cosmetic plant is rapidly gaining worldwide acceptance, and since it is widely grown in many parts of the world, this makes it readily available. This study was carried out to investigate some of the effects of its ethanolic root extract on the histology of the ovary of adult female wistar rats.

### **Methods**

Twenty-four adult female wistar rats were randomly distributed into 3 groups (n=8). Group A was given normal saline and this served as the control, Group B was administered 200mg/kg body weight, and group C was administered 600mg/kg body weight. These doses were administered once daily for 21 days, and the rats were sacrificed on the 22<sup>nd</sup> day.

### **Results**

The results showed there was an arrest in the development of the follicles in the ovary of the experimental groups and which was not observed in the control group.

### **Conclusion**

It can be concluded that ingestion of *Lawsonia inermis* has some deleterious effects on the ovaries investigated.

**Keywords-** *Lawsonia Inermis*, ovaries, follicular development, histology

## **INTRODUCTION**

Henna (*Lawsonia.inermis*) has been used cosmetically and medicinally for over 9,000 years. *Lawsonia inermis* is a popular skin and hair coloring agent in many parts of the world. Its root is considered a potent medicine for gonorrhoea and herpes infection. In addition, it is a medicinal plant traditionally used [1] by diverse groups of tribal/ethnic people [2,3,4] *Lawsonia inermis* is used as an antirheumatic and antineuralgic agent (Marc, 2008), and also has potential as an antidiabetic drug [4]. There is evidence of the plant having wound healing properties [5]. Furthermore, treatment with hydroalcoholic extract of *Lawsonia inermis* (*in vivo*) has been proved to increase levels of cellular antioxidant enzymes such as glutathione reductase, superoxide dismutase and catalase [6] . *Lawsonia inermis* extracts inhibit the oxidative damage pathway induced by hexavalent chromium and thereby prevent cell death. The plant can serve as a prospective source of natural phenolics and other metabolites which could prove to be precursors for designing effective drugs against heavy metal toxicity. Decoction of the root generally in combination with prepared indigo act as a powerful abortifacient [7]. Methanol extract of roots of *Lawsonia inermis* was most effective in inducing abortion in mice, rats and guinea pig [7]. The effect apparently was dosage-dependent. The results of whole animal experiments support the methanol extract effectiveness as an abortant due to its maternal and foetal toxic effects [8]. No study was found dealing with the effect of *Lawsonia inermis* on the ovary. This study therefore examine the effects of its ethanolic root extract on the histology of the ovary of adult female wistar rats.

## **MATERIALS AND METHODS**

### **Plant materials and Preparation of Extracts.**

Fresh plant samples (*Lawsonia inermis*) were collected from the Oja-Oba in Ilorin, Kwara state Nigeria. The samples were taken to the University Department of Plant Biology for authentication. The plant materials were washed with water, cut into pieces, sun dried for 2 days. The dried plant materials were then pulverized into coarse powder in a grinding machine. The powder was then soaked in absolute ethanol for 48 hours. The filtrate was then concentrated using a rotary evaporator.

### **Care and maintenance of animals**

The twenty-four adult female wistar rats were used in this evaluation and were acclimatised for a period of two weeks before this experiment in the Department of Anatomy, University of Ilorin Animal Holdings, University of Ilorin. All the animals were treated following the ethical guidelines of the laboratory animal committee of the College of Health Sciences, University of Ilorin. The rats were carefully assessed to be free of any pathological condition before starting the experiment by physical examination. They were housed in a standard light, temperature and humidity room in metabolic cages.

### **Experimental Protocol**

Twenty-four adult female rats weighing between 180-200g were used in this investigation. The experimental animals were randomly divided into three groups (A, B and C) of eight animals each. Group A served as control while the other two groups constituted the experimental groups. Group B was administered 200mg/kg body wt of the extract while group C was administered 600mg/kg body wt of the extract. The animals were sacrificed after 21 days of administration

### **Animal sacrifice**

The animals used were sacrificed via cervical dislocation and a midline incision on the anterior abdominal wall was made exposing the abdominal and pelvic viscera.

### **Histological preparation**

The ovaries were quickly dissected out and fixed in 10% formal saline for routine histological techniques. The tissues were dehydrated in an ascending grade of alcohol, cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thickness were obtained using a rotary microtome. The deparaffinized sections were stained routinely with haematoxylin and eosin. Photomicrographs of the desired sections were obtained for further observations.

## RESULTS

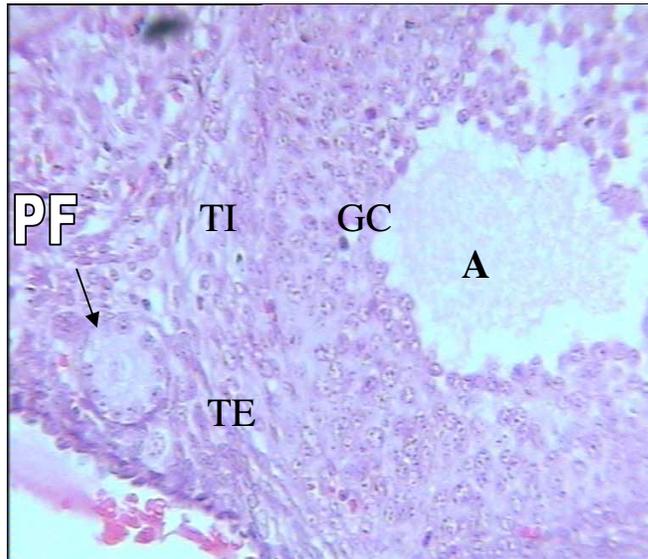


Figure 1-Photomicrograph of a section of the ovary of an adult female Wistar rat in group A (control) using H&E x400

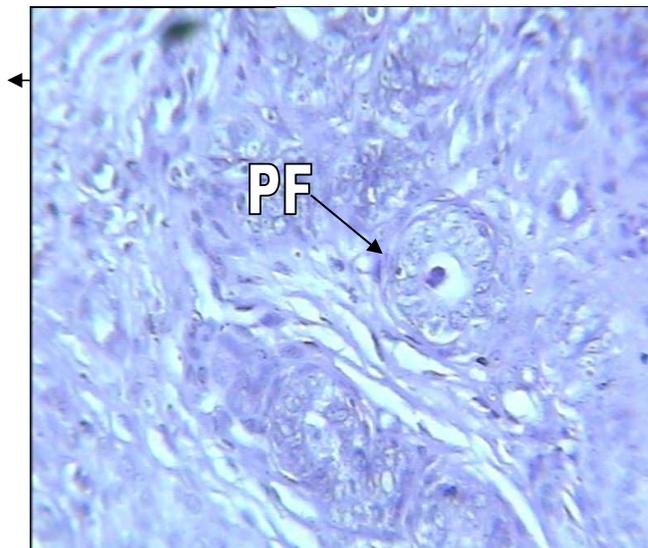


Figure 2 - Photomicrograph of a section of the ovary of an adult female Wistar rat in group B (200mg/kg body wt.) using H&E x400

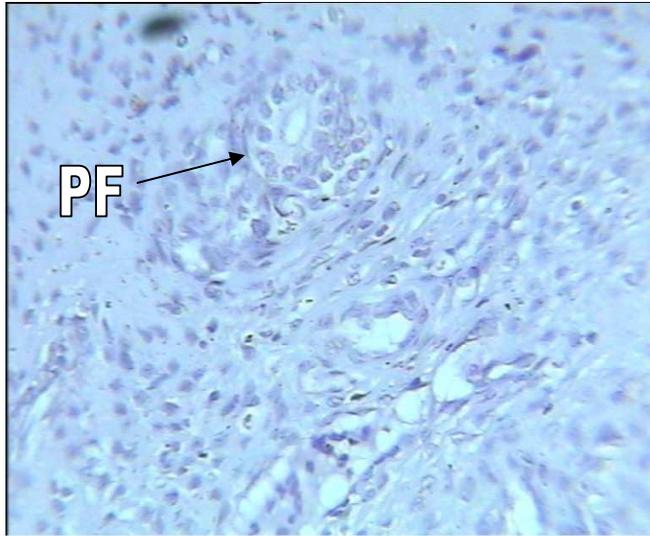


Figure 3. Photomicrograph of a section of the ovary of an adult female Wistar rat in group C (600mg/kg body wt.) using H&E x400

Legends to the keys in Figure 1,2 and 3

**KEYS**

PF: primary follicle

TI: theca interna

TE: theca externa

GC: granulosa cells

A: antrum

## **RESULTS**

Group A rats received normal saline and served as the control group. The histology as in Figure 1 shows follicles, at different stages of development. This is the regular pattern of a transverse section of the ovary.

Histology sections of the ovaries of rats in group B and C (Figure 2 and 3) have fewer number of developing follicles when compared to the control group, only the primary follicle were observed and no other stage of the developing follicle was seen and this arrest in development of the follicle could inhibit ovulation. This observation is dose-dependent.

## **DISCUSSION**

*Lawsonia inermis*, despite its wide cosmetic and medicinal usefulness has shown in this study to have follicular growth inhibitory effects. Even though *Lawsonia inermis* extracts inhibit the oxidative damage pathway and can serve as a prospective source of natural phenolics and other metabolites which could prove to be precursors for designing effective drugs against heavy metal toxicity [9], having anti-gonorrhoea antirheumatic, antineuralgic [3], and potential antidiabetic properties [4], it also possess a deteriorative effect at high dosages as seen in this study. As it is seen that histological sections of the ovaries of the rats in group B and C (Figure 2 and 3) have fewer number of developing follicles when compared to the control group A (Figure 1). Only primary follicles were observed and no other stage of the developing follicle was seen, thus this arrest in development of the follicle could inhibit ovulation. Ovulation inhibition will cause an anovulatory cycle in the animals and thus fertilization does not occur. It can serve as a potential contraceptive and remains to be seen whether it is reversible or not, as further studies on this is awaited.

The health risks involved in pre-mixed paste of *lawsonia inermis* can be significant. The United States [Food and Drug Administration](#) [10] considers these to be adulterants and therefore illegal for use on skin. Some pastes have been found to include: [silver nitrate](#), [carmine](#), [pyrogallol](#), disperse orange dye, and chromium [11]. These have been found to cause allergic reactions, chronic inflammatory reactions, or late-onset allergic reactions to hairdressing products and textile dyes [12, 13]. The use of henna in cosmetics should be strictly supervised or outlawed altogether.

Decoction of the root, generally in combination with prepared indigo act as a powerful abortifacient [7].Methanol extract of roots of *Lawsonia inermis* was most effective in inducing abortion in mice, rats and guinea pigs.

The observed abortifacient effect in other studies [7,8]might be due to inhibitory effect on the hormone secretions of the ovary at follicular, secretory and/ or progesterational phases, thus preventing the building up of the endometrial lining for pregnancy sustenance. This observed follicular growth inhibition might be due to inhibition of hypothalamic-hypophyseal axis hormone release or directly mediated effect on the integrity of the ovary.

Follicular growth inhibitory effects, in addition with the previously reported abortifacient effect of lawsonia inermis will probably aggravate infertility, as infertility is a worldwide problem affecting approximately 10-15% of couple [14] while the recent discoveries of the medicinal benefits of *Lawsonia inermis* will probably lead to an increased human use. Its cosmetic uses makes it more peculiar to women and this emphasises the importance of this experiment conducted on the ovaries of wistar rats, which are major organs in reproduction. Further work on the hormonal regulation with the administration of the extract is desirable to further confirm this observation.

It can be concluded from our findings that *Lawsonia inermis* has antifertility properties through suppression of the development of follicular cells of the ovaries.

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