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Effects of *Ficus asperifolia* on normal rat estrus cyclicity

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PEER REVIEW

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Comments

This study contributes to the understanding of the local effects of *Ficus asperifolia* upon the reproductive tract of females. The hypothesis of the authors was ascertained and results provided good evidence to support its traditional use in reproductive medicine of Cameroon folk. Other possibilities of medicinal use of *Ficus asperifolia* could be potentially analyzed with more precise techniques. Aims of the present study were covered but potential changes in vaginal smears should indicate subtle changes in vaginal cells that commonly indicate major changes in the periphery and induced by the plant fractions such as carotenes and alkaloids. I suggest its publication in the present form.
(Details on Page 56)

ABSTRACT

Objective: To evaluate *Ficus asperifolia* (Moraceae) (*F. asperifolia*) effecting on regular estrus cycle of Wistar rats. **Methods:** Air-dried fruits of *F. asperifolia* were extracted using water. Prior to the test, vaginal smear was monitored daily for a 3-week period to select females with normal (regular) estrous cycle. Those with regular estrus cycle weighing between 150-170 g were randomized into three sets of 15 animals each. Each set was then divided into three groups: Group 1 (control) was orally administered with distilled water (10 mL/kg body weight) once a day for 1 week starting from the proestrus stage. Groups 2 and 3 were respectively treated with 100 and 500 mg/kg body weight of the plant aqueous extract. The two other sets of 15 animals each were similarly treated as the first set for 3 weeks and 6 weeks respectively. Estrus cycle pattern was monitored before and during plant extract application whereas lipid profile, ovary, uterus and liver growth indices were determined at the end of each treatment. **Results:** *F. asperifolia* did not disrupt (0%) the order of appearance of normal estrus cycle stages, namely, proestrus, estrus, metestrus and diestrus. Short-term treatment (1 week duration) exhibited high frequency of appearance of proestrus and estrus stages while mid- (3 weeks) and long-term (6 weeks) treatments revealed constancy in the frequency of all stages irrespective to animal groups. The plasma and organ lipid profile, as well as ovary, uterus and liver growth remained unchanged when compared to distilled water-treated animals. Following long-term administration of plant extract (6 weeks), no adverse effect was noticed. **Conclusions:** Our data partially support the use of *F. asperifolia* in common medicine.

KEYWORDS

Ficus asperifolia, Estrus cycle, Rats.

1. Introduction

During preclinical investigations into the safety of drugs and chemicals, many are found to interfere with the reproductive function of the female[1,2]. This interference is commonly expressed as a change in normal components of vaginal smear or disruption in the frequency of particular stages of the estrus cycle[3]. In this light, alternative treatments with plant extracts are required to have more specific pharmacological profile[4,5]. The importance of plants as a source of fertility drugs has been emphasized by many researchers[6]. Fertility agents obtained from indigenous medicinal plants would be of immense benefit, especially to inhabitants of developing countries since the cost of these drugs would be within their means[7,8]. *Ficus*

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asperifolia (Moraceae) (*F. asperifolia*), known as "Ntchach lum" in the western region of Cameroon, is used to reverse some cases of sterility/infertility in women. Our earlier studies have shown that this plant, especially its aqueous extract, possesses real pro-implantation, pro-development, uterotrophic, and uterotonic-like activities[9,10]. These preliminary findings motivated us to find out whether an accidental intake of *F. asperifolia* by females with regular estrus cycle could be of any damage on their estrus cycle. In the present study, we examined the effects of a short-, mid-, and long-term oral administration of aqueous extract of *F. asperifolia* (the most efficient extract from our pilot study) on the regularity and frequency of appearance of rat estrus cycle stages.

2. Materials and methods

2.1. Plant collection and preparation of aqueous extract

Fresh fruits of *F. asperifolia* were collected during the month of February in 2011, from trees in Dschang, Cameroon. Botanical identification was performed in the Cameroon National Herbarium in comparison with the existing specimen number 338/15240/HNC. The fruits were shade-dried for 5 d and ground into powder. To obtain an aqueous extract similar to the traditional recommendation, a total of 1 kg of *F. asperifolia* were soaked in distilled water (5 L) and the mixture boiled for 15 min. The heated decoction was taken and allowed to cool at room temperature, filtered and oven-dried to give 46.67 g of dried aqueous extract (yield of extraction, 4.66%) used in the study.

2.2. Phytochemical screening

Qualitative phytochemical evaluation was performed on aqueous extract of *F. asperifolia* to determine the existence of sterol and triterpenes (Libermann Buchard test), flavonoids (test of Shinoda), and saponins (Hostettmann test)[11].

2.3. Animals

Healthy non-pregnant adult female Wistar rats between 10 and 12 weeks of age weighing 150-170 g were used in this study. They were housed in groups (five per group in polypropylene cages) and maintained under uniform husbandry conditions with natural photoperiod, humidity, temperature (26±2) °C and free access to food and water. The Local Committee of Ethics on Animal Experimentation approved all experimental procedures, which followed the regulations established by the European Union on Animal Care and Experimentation (CEE Council 86/609).

2.4. Experimental design

2.4.1. Estrus cycle monitoring

Three weeks prior to the treatment, vaginal smears were collected and observed each morning (8-10 a.m.) to determine estrus cyclicity of each animal. This involved sampling the cells of the vaginal canal with sterile saline using a glass pipette. The recovered solution containing cells was placed on microscope slides, fixed with methanol, stained with methylene blue, dried and examined microscopically. Cell descriptions were used to classify rats based on the stages of the estrus cycle (proestrus, estrus, metestrus and diestrus)[2].

2.4.2. Animal's treatment

A total of 45 females with regular estrus cycle were randomized into three sets of 15 animals each and treated for 1 (set I), 3 (set II) and 6 weeks (set III). Each set was then divided into three groups and treated as follows: Group 1 (control) was orally administered with distilled water (10 mL/kg body weight) once a day starting from proestrus stage. Group 2 and group 3 were respectively treated with 100 and 500 mg/kg body weight of aqueous extract of *F. asperifolia*. Estrus cycle pattern was monitored daily and the frequency of appearance of each stage determined. At the end of each treatment period, animals were euthanized under anaesthesia (diazepam/ketamine). Blood was collected by cardiac puncture and the plasma prepared and stored at -20 °C for biochemical analysis. Ovaries, uterus and liver were removed, blotted, weighed and kept at -20 °C.

2.4.3. Preparation of ovarian and uterus supernatants

Ovaries and uterus of each animal were homogenized in sterile saline at 1% and 5% respectively (0-4 °C) using a potter homogenizer. The homogenate was then centrifuged at 3 500 rpm for 15 min (Techmel & Techmel, USA). The supernatant was collected and kept frozen overnight at -20 °C before being used for various biochemical assays. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were determined in plasma, ovary and uterus using available commercial biochemical kits.

2.5. Statistical analysis

Data were expressed in mean±SEM. One-way ANOVA followed by post-hoc Fisher's LSD were used to analyze statistical difference among groups using STATISTICA, Statsoft, Inc. (2008), data analysis software system, version 8.0. www.statsoft.com. Comparisons with $P<0.05$ were considered to be statistically significant.

3. Results

3.1. Preliminary phytochemical analysis

The fresh aqueous and methanol extracts of *F. asperifolia* gave a positive reaction to alkaloids, saponins, sterols and triterpens.