Reproductive effects of *Ficus asperifolia* (Moraceae) in female rats

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Abstract

The reproductive effects of *Ficus asperifolia* in female rats were investigated in the present study. Sperm-positive adult female rats were orally administered (P.O.) either the aqueous and methanol extracts of Ficus asperifolia (100 and 500mg/kg), distilled water (10ml/kg) or 5%Tween 80 (10ml/kg) for seven days. On day 10 of pregnancy, the implantation sites were recorded. In the fertility study, adult female rats received the same test substances for 21 days and, the fertility index and litter size determined. In the uterotrophic test, normal and ovariectomized immature rats were treated for seven days with the dry extract of Ficus asperifolia (100 and 500mg/kg) in the absence and presence of 17â-estradiol benzoate 1µg/animal/day, s.c. On day 8, the uterine growth index was measured. Results of the study showed a significant increase ($p<0.05$) in the implantation sites and litter size of animals receiving 100mg/kg of the aqueous extract of Ficus asperifolia. In the estrogenic assay, normal immature rats were sensitive to the treatment with Ficus asperifolia than the ovariectomized ones. Our results give added scientific support to the popular use of Ficus asperifolia in the treatment of some cases of women's sterility/infertility related problems.

Keywords: *Ficus asperifolia*, implantation, fertility, uterotrophic, rat.

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Introduction

In developing countries, 80% of the population continues to use medicinal plants and plant products in handling primary medical problems due to their accessibility, availability and affordability. In these countries, a variety of plants are claimed to have fertility regulating properties and a few have been tested for such effect (Baker et al, 1999;Telefo et al, 2002; Ganguly et al, 2007; Cherdshewasart et al, 2007). *Ficus asperifolia* of the Moraceae family is one of these plants. It is a small or average size tree, terrestrial or epiphyte which can reach 20m in height. It is found in Senegal, Uganda, Tanzania, Natal (South Africa), Madagascar and Cameroon. *Ficus asperifolia* is abundant in the savannah regions, especially along river banks and marshy areas at an altitude of up to 1100m. The leaves are enormous and displayed spirally, the limb is largely oval or has a form of ellipse and the roots are most often fibrous (Adjanohoun et al, 1996). In the Western Province of Cameroon, this plant referred to as "Ntchach lum" or "Thutsia" is popularly used as shade-tree because of its large leaves and also in the building of hedges around houses. Traditional medicine of this same region indicates that the decoction of dry fruits of *Ficus asperifolia* is used to reverse some cases of sterility/infertility whereas the leaves are used as anthelmintic and purgative. Although there is no scientific evidence to support the ethnopharmacological reputation of *Ficus asperifolia* on female reproduction, tribes continue to popularly use it in the management of cases of sterility/infertility in women. The present work was therefore undertaken with an aim to scientifically validate this claim. As such, we evaluated the post-coital, fertility and uterotrophic potentials of the aqueous and methanol extracts from the dried fruits of *Ficus asperifolia* in immature and adult female rats.

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Methods

Plant material and preparation of extracts

Fresh fruits of *Ficus asperifolia* were collected in the month of February from trees in Dschang, Cameroon. Botanical identification was performed in the Cameroon National Herbarium (HNC) in comparison with the existing specimen number 338/15240/HNC. The fruits were shade-dried for 5 days

and ground into powder. Two types of extracts were used in the study. In order to obtain an aqueous extract similar to the traditional recommendation, 1kg of *Ficus asperifolia* was soaked in distilled water (5L) and the mixture boiled for 15 minutes. The heated decoction was taken and allowed to cool at room temperature, filtered and oven-dried to give 46.67g of dried aqueous extract (yield of extraction, 4.66%). To obtain the methanol extract, 1kg of *Ficus asperifolia* powder was soaked in 5L of methanol for 24h. The extract was filtered and the filtrate was evaporated to dryness at low temperature under reduced pressure in a rotary evaporator. Approximately 50g of dried methanol extract was obtained giving an extraction yield of 5%. For bioactivity investigations, the aqueous and methanol extracts were suspended in distilled water and 5%Tween 80 respectively. For each extract, the working solution was prepared at a final concentration of 100mg/ml in corresponding vehicle.

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of alkaloids, saponins, sterols and triterpens in the fruits (Klyne, 1970; Markham, 1982; Hostettmann et al, 1991; Bruneton, 1993).

Animals

Adult Wistar rats of either sex (> 3 months, 150-170g) and immature female rats (22 days old, 22-40g) were used in this study. They were fed with standard food and water *ad libitum* and maintained under laboratory conditions (temperature 26±2°C, 12h natural light/dark cycle). 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).

Experiments

Implantation study

Adult females used in this test were paired overnight with vigorous sexually experienced males. Successful mating was confirmed by the presence of sperm in the vaginal smear the following morning (07H:00-8H:00) and this day was considered as day 1 of pregnancy. Only sperm-positive females were used in the study. They were randomly partitioned into 6 groups of 5 animals each and treated as follows: Group 1: distilled water (10ml/kg, control 1); Group 2: 5%Tween 80 (10ml/kg, control 2); Groups 3-4: aqueous extract of *Ficus asperifolia* (100 and 500mg/kg); Groups 5-6: methanol extract of *Ficus asperifolia* (100 and 500mg/kg). The extracts and vehicles were given P.O. for seven days. On day 10 of pregnancy, each female was laparatomised under diazepam/ketamine (10/50mg/kg respectively) anaesthesia and the number of implantation sites counted.

Fertility study

In order to determine the long term administration effect of *Ficus asperifolia* on the fertility of sexually naive female rats, thirty primipare adult animals were used and divided similarly as in the implantation test. They were orally treated with the test substances for 21 consecutive days. During the last five days of treatment (Day 17 - Day 21), each female was allowed mating with a vigorous male of proven fertility. The vaginal smear was daily examined under microscope and the sperm-positive females were isolated and followed-up until parturition. During testing, the rats were weighed daily in order to monitor weight gain. At the end of the study, the litter size was recorded and the fertility index calculated using the following formula: fertility index = (number of pregnant / number of mated) x 100 (Ratnasooriya and Dharmasiri, 2000).

Uterotrophic assay

Gonado-intact (n=30) and ovariectomized (n=30) immature females were used. Ovariectomy was performed as previously described (Watcho et al, 2007). Among the two extracts of *Ficus asperifolia*, the aqueous extract showed maximum changes in the previous experiments and was thus used in this investigation. Each category of rats was randomly divided into 6 groups of 5 animals and treated for seven consecutive days with one of the following: distilled water (10ml/kg, vehicle control, orally); aqueous extract of *Ficus asperifolia* (100 and 500mg/kg, orally); 17b-estradiol benzoate (Sigma Chemicals, USA) (1mg/rat, positive control; s.c.); the sequential treatments with aqueous extract (100mg/kg) plus estradiol (1mg/rat) and aqueous extract (500mg/kg) plus 17â-estradiol (1mg/rat, s.c.). During testing, the body weight of each animal was recorded daily and the weight gain determined. Animals of all groups were sacrificed under ether anaesthesia 24h after the final dose. Uteri were excised, trimmed free of any fat and adhering non-uterine tissue. The body of the uterus was cut just above its junction with the cervix and at the end of the uterine horns with the ovaries. The uterus relative weight was calculated as follows:

Relative weight of the uterus (mg/100g) = [uterus wet weight (mg) / body weight (g)] x 100. The uteri weight was used as an index of uterine growth (Odum et al, 1997; Baker et al, 1999).

Statistical analysis

Data are expressed in mean ± SD. One-way analysis of variance (ANOVA) followed by post-hoc LSD was performed using SPSS for Windows version 10.0. Comparisons with p values < 0.05 were considered to be statistically significant.

Results

Effects on implantation

Table 1 summarises the results of the post-coital administration of the aqueous and methanol extracts of the dried fruits from *Ficus asperifolia* on adult female rats after seven days of treatment. When compared to respective control, a tendency to increase in the number of implantation sites was observed throughout the study with significant ($p<0.01$) effects recorded in females receiving the aqueous extract (100mg/kg) of *Ficus asperifolia*.

Effects on fertility

All plant extract-treated animals showed a non significant increase in the body weight after 21 days of continuous drug administration when compared to respective controls. However, females receiving 100mg/kg of aqueous extract were the most sensitive to the treatment with an increase of 13.30% in the body weight and a significant ($p<0.01$) higher number of pups at parturition. The fertility index was not affected in all groups (Table 1).

Table 1: Effects of aqueous and methanol extracts of Ficus asperifolia ***on implantation and fertility of adult female rats.***

Treatment	Implantation		Fertility		
	Number of sperm-positive females	Number of implantation sites	Body weight gain (%)	Fertility index (%)	Mean litter size
Distilled water (10ml/kg)	5	4.20 ± 1.16	9.05 ± 4.43	100.00	7.50 ± 0.75
Aqueous extract (100mg/kg)	5	7.20 ± 0.58*	13.30 ± 4.88	100.00	10.40 ± 0.31**
Aqueous extract (500mg/kg)	5	5.60 ± 1.54	9.78 ± 1.98	100.00	5.80 ± 0.27
5% Tween 80 (10ml/kg)	5	5.00 ± 0.32	10.11 ± 1.95	100.00	7.00 ± 0.31
Methanol extract (100mg/kg)	5	5.60 ± 0.68	12.43 ± 4.04	100.00	7.00 ± 0.26
Methanol extract (500mg/kg)	5	5.20 ± 0.73	10.25 ± 4.05	100.00	6.00 ± 0.80

Number of rats in each group = 5

*: $p<0.05$ and **: $p<0.01$ significantly different compared to control 1 (distilled water)

Uterotrophic effects

In all groups of rats, the body weight was increased and remarkably expressed in females treated either with 100mg/kg of aqueous extract of *Ficus asperifolia* ($p<0.01$) or with the combined treatment of aqueous extract of *Ficus asperifolia* (100mg/kg) plus 17β-estradiol benzoate ($p<0.001$). A general increase ($p<0.001$) of the uterine weight was noticed when compared to distilled water. The highest uterotrophic effect recorded in this study was obtained in animals receiving the combined treatments of 17β-estradiol benzoate and plant extracts in a dose-dependent manner (Table 2).

As observed in normal females, the body weight of ovariectomized immature rats treated with the aqueous extract of *Ficus asperifolia* was also increased after seven days of drug application with the maximum body weight gain being 22.48% in females treated with 100mg/kg of aqueous extract. The uterus growth was significantly elevated ($p<0.01$ - 0.001) after *Ficus asperifolia* (500mg/kg), 17β-estradiol benzoate (1µg/rat/day) and the various sequential treatments. It's noteworthy mentioning that the sequential treatment with *Ficus asperifolia* (500mg/kg/day) plus 17β-estradiol benzoate (1µg/rat/day) was the most effective in the study (189.45% and 26.86% of increase compared to distilled water and estradiol benzoate groups respectively). Results in Table 2 also showed that at equivalent dose, the uterotrophic effects of *Ficus asperifolia* was more expressed in gonado-intact rats than in ovariectomized category.

Discussion

The aim of the present study was to evaluate the implantation, fertility and uterotrophic properties of the aqueous and methanol extracts of the dried fruits from *Ficus asperifolia*, a medicinal plant with ethnopharmacological reputation in the treatment of some cases of sterility/infertility in women.

Post-coital administration of *Ficus asperifolia* extracts for seven consecutive days to sperm-positive

Table 2: Effects of aqueous extract of *Ficus asperifolia* on body weight and relative weight of uterus of immature gonado-intact and ovariectomized rats after 7 days of treatment.

Treatment	Gonado-intact		Ovariectomized	
	Body weight gain (%)	Uterus (mg/100g)	Body weight gain (%)	Uterus (mg/100g)
Distilled water (10ml/kg, control) (n=5)	2.47 ± 0.90	126.19 ± 18.06	9.57 ± 3.67	93.59 ± 21.29
Ficus asperifolia (100mg/kg) (n=5)	8.91 ± 1.51**	147.57 ± 27.95***[d]	22.48 ± 4.17**	86.65 ± 9.34[b]
Ficus asperifolia (500mg/kg) (n=5)	6.68 ± 0.72[a]	167.54 ± 10.03***[b]	19.44 ± 2.98*	110.82 ± 40.00[a]
Estradiol benzoate (1mg/kg) (n=5)	5.40 ± 2.24	292.47 ± 27.15***	40.11 ± 17.72***	213.55 ± 36.39**
Ficus asperifolia (100mg/kg) plus Estradiol benzoate (1mg/kg) (n=5)	5.97 ± 1.06	358.63 ± 37.89***	12.44 ± 4.84[a]	215.16 ± 44.96**
Ficus asperifolia (500mg/kg) plus Estradiol benzoate (1mg/kg) (n=5)	10.42 ± 2.01***[a]	368.20 ± 31.25***[a]	7.29 ± 1.39	270.90 ± 36.80***

Number of rats in each group = 5. **: $p<0.01$ significantly different compared to control 1 (distilled water)
[a]: $p<0.05$, [b]: $p<0.01$ and [d]: $p<0.001$ significantly different compared to estradiol benzoate

female rats did not provoke any failure of implantation. Rather, a tendency to increase the number of implantation sites was noticed especially in animals treated with 100mg/kg ($p<0.05$) of the aqueous extract. This result implies the induction by the plant extract components, of a favourable milieu for zygote implantation and development. Implantation is a crucial event in mammalian embryonic growth and development which occurs 4-6 days after fertilization in human and rodents. It is regulated by a timely interplay of the ovarian hormones (estrogens and progesterone) and any disturbance in the equilibrium level of these hormones may lead to loss of implantation and may cause infertility (Ding et al, 1994; Hiremath et al, 1999). It has also been reported that conceptus synthesis and release of estrogens is essential for establishment of pregnancy and, endometrial exposure to estrogen prior to the normal conceptus secretion results in total pregnancy loss (Gries et al, 1989; Ashworth et al, 2006; Ross et al, 2007). In the present work, it could then be suspected that *in vivo* exposure of the uterus to *Ficus asperifolia* extracts for seven days may have contributed to the set up of some modifications in the uterine endometrium that transform it from a non receptive to a receptive phase allowing the implantation and development of the blastocyst. In addition, the increase in litter size after exposure of the reproductive system to chronic administration of *Ficus asperifolia* extracts (21 days of treatment, fertility test) further denotes an uterotrophic effect of *Ficus asperifolia*.

In an attempt to clarify this suspected uterotrophic activity, the immature rat uterotrophic assay (Wakeling et al, 1991; Baker et al, 1999), one of the most widely used methods to detect estrogenicity, was carried out. Administration of the aqueous extract (the most active in the above experiments) of *Ficus asperifolia* to immature normal rats resulted in an increase of the uterine weight. This result confirms the uterotrophic-like effect of the bioactive compounds contained in the aqueous extract of this medicinal plant.

Preliminary phytochemical screening has revealed the presence of sterols in the aqueous extract of *Ficus asperifolia* which may account for the reported activity. Indeed, many studies have suggested that phytosterols may have effects on the reproductive system and in particular that they possess estrogenic activity (Rosenblum et al, 1993; Mellanen et al, 1996). In order to monitor and ensure the performance of the test system and to provide some reference data, 17β-estradiol benzoate was included in the uterotrophic test. As expected, this positive control 17β-estradiol benzoate (1μg/rat) significantly increased ($p<0.01$-0.001) the uterus weight compared to females orally treated with distilled water (10ml/kg) and plant extracts (Hiremath et al, 1999). However, the uterotrophic response was more greater after the sequential administration of 17β-estradiol benzoate (s.c.) and aqueous extract at all doses

used. These findings clearly indicate a dose-dependent potentiating or facilitating effect of *Ficus asperifolia* on estrogen activity in immature female rats. Interestingly, results of the uterotrophic assay show a high sensitivity of gonado-intact females to the various treatments compared to ovariectomized rats. This view, which implies a potentiating effect of *Ficus asperifolia* on endogenous estrogen's activity, matches with some reported data in the literature (Baker et al, 1999) and further supports the suspected hypothesis of pro-implantation and pro-development properties proposed above. Further studies on the rat isolated estrogenized and non estrogenized uterus will help to complete and better ascertain these potentiating effects of *Ficus asperifolia*.

Considering all these findings, it could be concluded that *Ficus asperifolia* possesses real pro-implantation, pro-development and uterotrophic-like activities at the doses used and, the aqueous extract had greater activity than the methanol extract. These results also give value to the popular use of *Ficus asperifolia* in handling some women's sterility/infertility problems.

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