

Original Article

Relationship of anti-fertility effects of *Andrographis paniculata* and hormonal assay in female rats

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Abstract

Aims: This study was aimed to find out anti-fertility effects of *Andrographis paniculata* (AP) plant. **Study design & Methodology:** A prospective case control animal study with 85 female and 30 male rats (Total 115 rats) was done in the Department of Pharmacology of Dhaka Medical College and Bangladesh Medical College, Dhaka, Bangladesh from July 2002 to December 2003. The total 85 female rats were grouped into case study or exposure group (N=60 female rats) exposed to water extract of AP & control or non exposure group (N=25 female rats, 30 male rats also considered as control group) Exposure group (water extract) of female were again subdivided in 3 groups according to duration of (A.P) exposure (N=20 in each group) e.g. 4 weeks, 6 weeks and 8 weeks. Mating schedules were done after the completion of scheduled duration of exposure with A.P (Dose was 1 gm/kg). **Results:** In group I percentage of infertility was 33.33% in 4 weeks exposure, 50% in 6 weeks exposure & 100% in 8 weeks exposure respectively. In control group (Group II) percentage of infertility was 0%. In case study group of female rats the value of FSH was 1.20 IU/L (4 weeks), 1.12 IU/L (6 weeks), 1.00 IU/L (8 weeks), LH 0.78 IU/L (4 weeks), 0.70 IU/L (6 weeks), 0.64 IU/L (8 weeks), Estrogen 45.30 pg/ml (4 weeks), 44.80 pg/ml (6 weeks), 44.20 pg/ml (8 weeks) and Progesterone 4.84 nmol/L (4 weeks), 4.72 nmol/L (6 weeks) and 3.80 nmol/L (8 weeks). In non exposure group the value of FSH was 1.23 IU/L, LH 0.80 IU/L, Estrogen 47.05 pg/L and Progesterone 5.50 nmol/L. In exposure group all the values were lesser than the normal hormonal value. **Conclusion:** This study suggests that due to lower level of hormone, female rats have promising percentage of infertility with AP. Further study is needed with rat as well as clinical trial with human being.

Key Words: *Andrographis paniculata*, Antifertility effects

Introduction

The scientists all over the world are putting much emphasis on herbs and plants in the fertility control. Herbal drugs are usually effective, inexpensive & afforded by people of rural area^{1,2,3,4,5}. Bangladesh is rich in natural resources of herbs. *Andrographis Paniculata* (AP) also known as king of bitters is a member of the plant, family Acanthaceae and has been used for centuries in Asia to treat various ailments including GI tract and upper respiratory infections, fever, sore throat and a variety of other chronic and infectious diseases for prevention of contraception^{6,7,8,9}. The leaves contain the highest amount of andrographolide (2.39%), most active component with very bitter taste & colorless crystalline in appearance^{10,11,12,13,14}. According to recent research in human being *Andrographis* are highly bioavailable and

readily absorbed from G.I.T. 80% of andrographolides are excreted by kidney within 8 hours^{15,16,17,18}. In animals there is no acute or short time toxicity in very high doses (1 – 10 gm/kg body weight) & no effects on major organs^{19,20,21}. The antifertility effect has been evaluated in the screening programme done by Zoha *et al* in 1989 with both male and female mice & confirmed the antifertility effect^{22,23}. One study also done by Jean barilla in 1999 with female mice and also shown the promising anti fertility effect & significantly lowers all the hormonal value such as follicle stimulating hormone (FSH), leutinizing hormone (LH), estrogen, progesterone & testosterone^{24,25,26}. We have proposed the study to investigate the antifertility effect of *Andrographis paniculata* with an aim to find out a safe, acceptable and effective method of contraception of female rats.

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Materials and Methods

This study was planned to design as a prospective case control study and was carried out to find out any association between *Andrographis paniculata* plant and hormonal assay related to antifertility effects in female rat and was done in the Department of Pharmacology, Dhaka Medical College and Bangladesh Medical College. The duration of study was 18 months. The healthy rat weighing 120 – 150 gm, age between 45 – 60 days was selected as study population. The mating outcome and its effects on fertility (Pregnancy) were observed. Serum FSH, LH, estrogen and progesterone were determined. The group exposed to water extract of AP was considered as case study group & non exposure of AP group was considered as control group. The exposure group was again subdivided into 3 subgroups according to duration of exposure of *Andrographis paniculata* such as 4 weeks (n=20), 6 weeks (n=20) & 8 weeks (n=20).

After completion of exposure with AP (4 weeks, 6 weeks and 8 weeks) female rats were kept into different cages and labeled properly and allowed to take normal diet. In group-I 20 treated female rats from each group and 6 untreated male rats from control group were taken. In group-II 25 untreated female rats and 12 untreated male rats were taken. Then mating outcome has been observed. They have kept together until 3 times birth of litters of control group & in this experiment they have kept together for 3 months because usually rats gave birth of litter 3 times within the 3 months. Then number and percentage of pregnancy and percentage of infertility (%) have been observed. The evidence of pregnancy was observed daily for a period of one week. Hormonal assay such as LH, FSH, Estrogen and Progesterone for female rats in exposure groups (4weeks, 6weeks & 8 weeks) & also in non exposure group were done to observe the anti-fertility effects.

Results

Table I – Distribution of study population

Total no of rat population	Sex	No of Population	Case study group (Exposure group)	Control group (Non exposure group)
115	Female	85	60	25
	Male	30	-	30

Table II – Distribution of study population according to duration of exposure of *Andrographis paniculata* (4 weeks, 6 weeks and 8 weeks)

Group	Preparation of AP	Duration of Exposure	Total No. of Female Rat
Case study group (Exposure group)	Water Extract	4 weeks	20
		6 weeks	20
		8 weeks	20
		Total	60

Table III– Mating schedule and rat population according to duration of exposure (both male and female rat)

Total Population	Preparation of AP	Sub Group	Duration of Exposure		
			4 weeks No.	6 weeks No.	8 weeks No.
115	Water extract	Group I TF +UTM	20 + 6	20 + 6	20 + 6

Table IV – Outcome of mating behavior (percentage of infertility)

Group	Preparation of AP	Sub Groups	% of infertility			
			Duration of Exposure			
			4 Weeks	6 Weeks	8 Weeks	
Case study group	Water extract 0.8 mg/g	Group I	UTM (18) + TF (60)	33.33%	50.00%	100.00%
		Group II	UTM(12) + UTF (25)	No exposure (AP)		
				0%	0%	0%

Table V – Hormonal assay (FSH, LH, Estrogen and Progesterone) of female rat of both exposed and non exposed group (Water Extract)

Group	Sex	AP Preparation	Duration of exposure of AP	Hormonal Assay			
				FSH IU/L	LH IU/L	Estrogen pg/ml	Progesterone on nmol/L
Exposed group	female Rat	Water Extract	4 Weeks	1.20	0.78	45.30	4.84
			6 Weeks	1.12	0.70	44.80	4.72
			8 Weeks	1.00	0.64	44.20	3.80
Control Group		No exposure of AP		1.23	0.80	47.05	5.50

Results showed percentage of infertility (%) in 4 weeks, 6 weeks and 8 weeks are 33.33%, 50.00% and 100% respectively and also showed the values of FSH, LH, estrogen, progesterone of exposure group were lesser than the control group.

Discussion

This prospective case control animal study was done to observe the antifertility effect of female rats with *Andrographis paniculata* by assessment of hormone. This study includes 85 female rats & 30 male rats and who have exposed to water extract of AP considered as case study or exposure group n= 60 female rat) and who have not taken watery extract of *Andrographis paniculata* were considered as control group n= 25 (female) + 30 (male). Exposure groups (watery extract) of female rats were again subdivided into 3 groups according to duration of *Andrographis Paniculata* (AP) exposure (n = 20 in each group) Like 4 weeks, 6 weeks and 8 weeks. After completion of scheduled duration of exposure with *Andrographis Paniculata* (AP), exposed female rats & non exposed male rats

were kept together for mating. Dose of AP was 0.8 mg/ml. In group I. percentage of infertility was 33.33% in 4 weeks exposure, 50% in 6 weeks exposure & 100% in 8 weeks exposure respectively. In control group (Group II) percentage of infertility was 0%. In our country 3 studies have been done by Zoha *et al* with the doses of 2 gm/Kg body weight of sun dried *Andrographis paniculata* powder in both male & female mice everyday for 6 weeks. None of the animals were pregnant after mating. In that study, population was less so in this study large number of study population was taken. In case of female rat percentage of infertility was 33.33% in 4 weeks, 66.67% in 6 weeks and 100% in 8 weeks exposure with *Andrographis paniculata*. So in this study it has been also observed that percentage of infertility increases with duration of exposure. In case group of female rats the value of FSH was 1.20 IU/L (4 weeks), 1.22 IU/L (6 weeks), 1.00 IU/L (8 weeks), LH 0.78 IU/L (4 weeks), 0.70 IU/L (6 weeks), 0.64 IU/L (8 weeks), Estrogen 45.30 pg/ml (4 weeks), 44.80 pg/ml (6 weeks), 44.20 pg/ml (8 weeks) and Progesterone 4.84 nmol/L (4 weeks), 4.72

nmol/L (6 weeks) and 3.80 nmol/L (8 weeks). In non exposure group the value of FSH 1.23 IU/L, LH 0.80 IU/L, Estrogen 47.05 pg/L and Progesterone 5.50 nmol/L. In exposure group all the value are lower than normal hormonal value. Yin J and L Guo in 1993 in Beijing cultured human placental tissue showed that andrographolide sodium succinale (derived from AP) was effective in inhibiting human progesterone production. Present study strongly confirms the findings of the above study. In this study progesterone was inhibited. The level of estrogen was also decreased in exposure group in comparison to non exposure group. It has been suggested that with exposure to *Andrographis paniculata* ovulation may be inhibited because hormonal level of LH, FSH, estrogen & progesterone were decreased in female rats. This study suggests that female rats have promising percentage of infertility with *Andrographis paniculata*. Many studies have been done in different countries in the world and found *Andrographis paniculata* was extremely

nontoxic even in higher doses. In our study higher dose has given and no toxicity has been reported. In Japan and America and other countries of the world many clinical trials have been done and found extremely nontoxic successful results. Meanwhile American investigators obtained investigational new drug status for the FDA to test *Andrographis paniculata* extract. Further clinical trial with human being is needed because of its little or no significant side effect even in very high dose to human and animal model and having very satisfactory antifertility effect, especially in female rats observed in our study. So *Andrographis paniculata* is may recommended to be tested clinically as herbal antifertility agent.

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