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Phytochemical analysis and fertility enhancement effects of aerial parts of *Fagonia arabica* in male and female rats

Asma Wazir[1], Nudrat Fatima[1], Iqra Kanwal[1], Darakhshan Masroor[2], Mehwish Khan[1], Mehreen Zaheer[1] and Atia Tabassum[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan
[2]Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

Abstract: In this study, fertility enhancing effect of ethanol extract of aerial parts of *Fagonia arabica* was evaluated. 24 female and 12 male virgin Wistar rats (Average wt 150-250g) were selected. Dose was given daily for 23 days in 4 groups of animal. After 23 days, they were cohabitated for mating. Then dosing was continued for further 5 days. On 5th day of mating, sperm counting was done by observing vaginal smear under the microscope which showed fertility enhancement in male. Female were kept separately until delivery. The number of pubs born provided female fertility enhancement as compared to control. Average sperm count in 1 cm^2 were counted in group A, B, C and control as 14.000±1.732, 12.000±1.000, 23.333±1.528 and 11.000±1.000 respectively. Number of pubs were counted in Group A, B, C and D as 8.667±2.082, 7.333±1.528, 7.333±1.528 10.000±2.000 and 5.333±0.577 respectively and fertility index was calculated for Group A, B and C as 62.516%, 37.502% and 87.512% respectively. Result showed that the orally administered dose of *Fagonia arabica* possess highly significant fertility enhancing activity in male and female rats after observing improvement in the sperm count and number of pubs as compared to control.

Keyword: *Fagonia arabica,* fertility, mating, sperm count, number of pubs.

INTRODUCTION

Infertility is a growing problem which affects the life of married couples badly (Anoky *et al*., 2017). Diverse factors are responsible for female infertility like endometriosis, polycystic ovary syndrome, oxidative stress, obesity etc. (Gambineri *et al*., 2019). Low quality and count of sperms are related to male infertility which is mainly due to heredity and hormonal level (Precone *et al*., 2021). Other factors which further contribute to infertility are the drugs used in treatment of diseases (diabetes and hypertension), environmental toxins, pesticides, heavy metals and delayed age marriages (Silvestris *et al*., 2019).

Various treatment options were used accordingly to treat problems related to male and female infertility. Male infertility can be treated by drugs, surgery and assisted reproductive technology which improve the reproductive problems and heal up the infections and consequently improve the sperms count. Surgery, medication, IVF and intra uterine insemination (IUI) were used to treat female infertility. Natural remedies rich in antioxidants are helpful to improve sperm count and sperm motility. Minerals and vitamins such as iron, zinc, vitamin C and vitamin E are helpful to resolve the issues related infertility. Number of herbal drugs are used to treat hormonal imbalance, spermatogenesis, sperm count, sperm motility and different female infertility causing problems (Healthline.com, 2020; Mayo Clinic.org).

Fagonia arabica L., belongs to family Zygophyllaceae. Its local name is dhamasa booti. It is a shrub and found in deserts and hilly areas of South Asia, Middle East, Central Europe, North Africa, California and Chili (Shinwari *et al*., 2007; Puri *et al*., 2014). Different active constituents are reported in *F. arabica* plant such as Glycosides, Flavonoids, Triterpenes, Saponins, Sulfur etc. Flavonoid glycosides of *F. arabica* are important phenolic compounds which are responsible for antioxidant activity (Tawaha *et al*., 2007). Species of genus *Fagonia* have exhibited numerous pharmacological activities including anti-inflammatory, anti-allergic, neuroprotective, androgenic, endocrinological, antimicrobial, cytotoxic, analgesic, antioxidant, hepatoprotective and antihemorrhagic (Puri *et al*., 2014; Farheen *et al*., 2017). *Fagonia arabica* reported as traditional medicine and have blood purifying properties. It also important for their anticoagulant effect and mollucidal activities (Chourasia *et al*., 2013; Gholkar *et al*., 2020). It also effective against myocardial infarction and significantly reduce blood clots in vessel (Das and Kaushik, 2010).

Adverse effects of various modes of infertility medication, failure of assisted reproductive techniques (ART) and other measures which helpful in conception lead to different psychological problems in childless couples (Zagami *et al*., 2019). Phytomedicines have efficacy to resolve various problems which cause difficulties in reproduction such as weakness in sexual activities, menstrual problems, infections and also reduce the risk of abortion (Aziz *et al*., 2018). Few phytomedicines work synergistically with reproduction techniques in treatment of infertility (Lans *et al*., 2018). In

**Corresponding author:* e-mail: asmanizam77@yahoo.com

recent study, fertility enhancement activity of *Fagonia arabica* was evaluated in both male and female rats. As reported in literature, presence of phenolic constituents is responsible for antioxidant properties in the extract of *F. arabica*. Strong evidence supports the association between the reactive oxygen species and the causes of male and female infertility. *F. arabica* may be beneficial for treatment of infertility due to its antioxidant properties (Satpute *et al*., 2009; Nath and Roy, 2021)

MATERIALS AND METHODS

Collection of plant and authentication

Aerial parts of *Fagonia arabica* was purchased from local herbal supplier and authenticated from herbarium by Department of Pharmacognosy, University of Karachi, Pakistan. A voucher specimen No FAH-01-19 was issued.

Preparation of extract

The material of plant was grinded into fine powder form with the help of grinder and 1 Kg of plant material was soaked in 10 folds of ethanol at room temperature for 7 days. Consequential extract was filtered and solvent was evaporated at temperature 60°C in rotary evaporator under pressure. After that the extract was dried in fume hood and used for experimental work.

Phytochemical analysis

Phytochemical studies were performed for the detection of Tannins, Phenols, saponins, alkaloids, terpenoids, carbohydrates, flavonoids, steroids and anthraquinones according to the standard reported methods (Syed *et al*, 2013; Gul *et al*, 2017)

Experimental animals

Wistar Albino rats of both sexes, weight range 150gm to 250g, were procured from animal house, Faculty of Pharmacy, Jinnah University for Women, Karachi. Both sexes of rats were kept separately in quarantine for a period of two weeks with unrestricted food and water. Screening of animals was done continuously for the selection of healthy, alert and nulliporous rats to conduct the mating trial test.

Experimental protocol for the evaluation of the fertility enhancing effect of ethanol extract of *Fogonia arabica* on male and female Wistar rats was approved by Institutional Ethical Committee of Jinnah university for Women (Ref letter No. JUW/Pharm/Ethic/01-2020)

Acute oral toxicity

Aqueous extract of *Fagonia arabica* was administered orally at different doses i.e. (5, 50, 300 and 2000 mg/kg p.o.) to four separate groups of animals. Each group contained 3 animals. The behavior variation and death rate were noted for 24 hours. Animals were also additionally observed for further 3 days for any fluctuation in behavioral changes, pupil extention, secretion, elimination and late deaths. No death was detected in period of 3 days (OECD Guideline for testing of chemicals, 2008; Wikipedia, 2021).

Evaluation of fertility

Mating trial test

A total of 36 virgin Wistar rats (24 female and 12 male) weighing between 150-250g were selected for the study. The rats were kept in quarantine for a period of 2 weeks. Food and water were removed 24 hours before the experiment. 50 mg/kg oral dose of *Fagonia arabica* ethanol extract was given to male and female rats of each group according to the scheme provided below for a period of 23 days. Distilled water was administered orally to control group of male and female rats.

Fertility assay was conducted in 4 groups of animal, each group contained total 9 animals (6 female and 3 male in which male and female were kept in separate cages) and the test drug was provided as follows:

Group A (Test group 1) - Only males were treated;
Group B (Test group 2) - Only females were treated,
Group C (Test group 3) - Both males & females were treated
Group D (Control group) - Both male & female were untreated

Mating trial test was carried out on 23rd day of administration of test drug. Animals were cohabitated for 10 days with a ratio of 1 male: 2 female and kept in separate cages.The dosing was continued till 27th day of experiment i.e. further 5 days after mating. Positive mating was confirmed by vaginal smear test. The vaginal fluid was taken and observed under microscope for sperm count. No. of sperms counted during mating trial test provided male fertility enhancement. Females were kept under observation and the resultant pregnancies were noted until birth.The no of litters born provided female fertility enhancement (Shah *et al*, 2016).

RESULTS

Phytochemical analysis

Results of phytochemical tests showed that tannins, phenols, saponins, alkaloids, terpenoids, steroids, carbohydrates, glycoside and flavonoids were present while anthraquinones were absent (Table1) in the test sample i.e. ethanol extract of *Fagonis arabica*.

Acute oral toxicity

After performing the test all animals were healthy and alert and no mortality was recorded which shows that the test drug is non toxic and can be used orally.

Fertility assay

The fertility enhancing activity was conducted as discussed in experimental section. On the 5th day of mating, sperms counting were done.

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Table 1: Phytochemical analysis of *Fagonia arabica* ethanol extract

Tannin	Phenol	Saponin	Alkaloids	Terpenes	Steroids	Carbohydrates	Glycosides	Flavonoids	Anthraquinone
Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative

Table 2: Evaluation of fertility

Groups	Average Sperm Count	No. of Litters	% Fertility
A	14.000±1.732*	8.667 ± 2.082*	62.516%
B	12.000±1.000	7.333 ± 1.528	37.502%
C	23.333±1.528*	10.00 ± 2.000*	87.512%
D	11.000±1.000	5.333 ± 0.577	-------

Data presented as Mean± S.E.M; Significant (*= Significant results at P≤0.05)

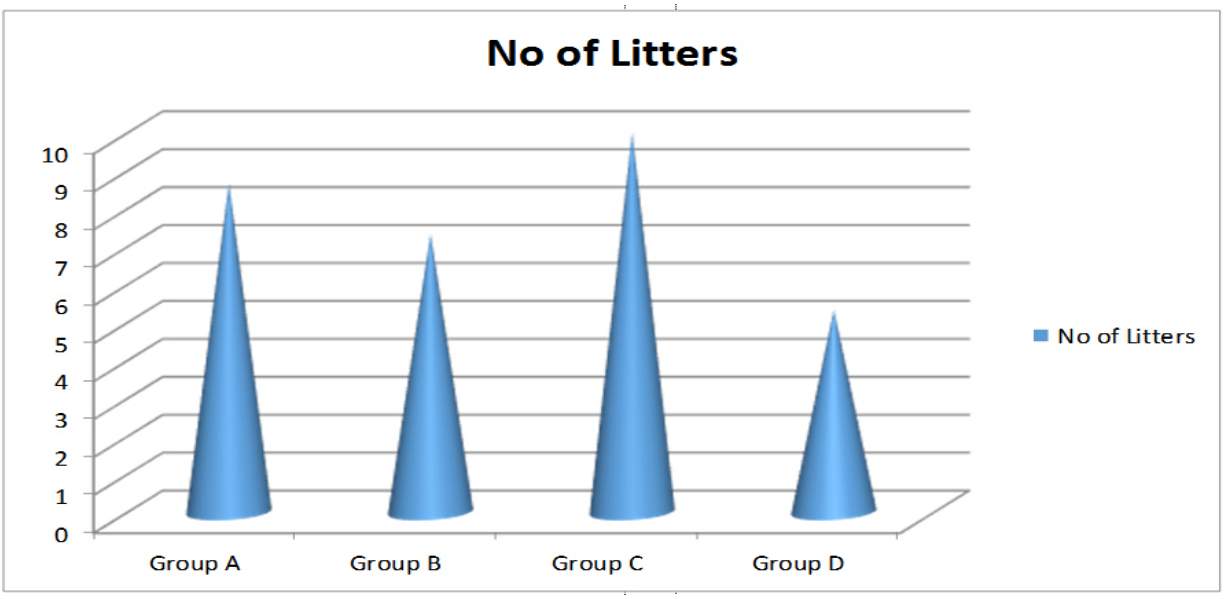

Fig. 1: Fertility activity of *Fagonia Arabica*

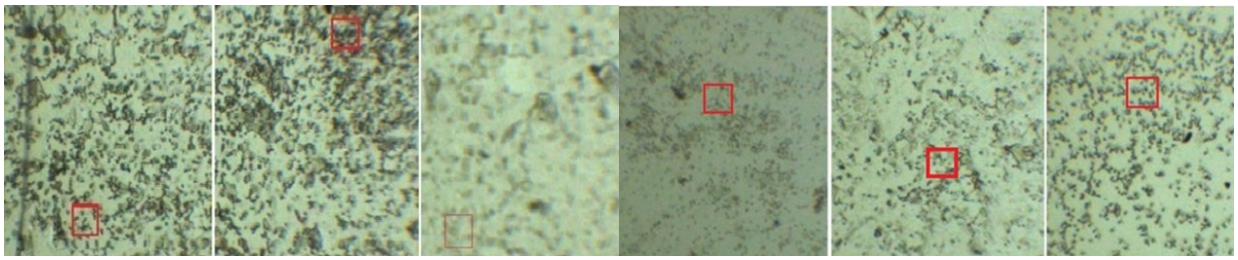

Fig. 2: Group A

Fig. 3: Group B

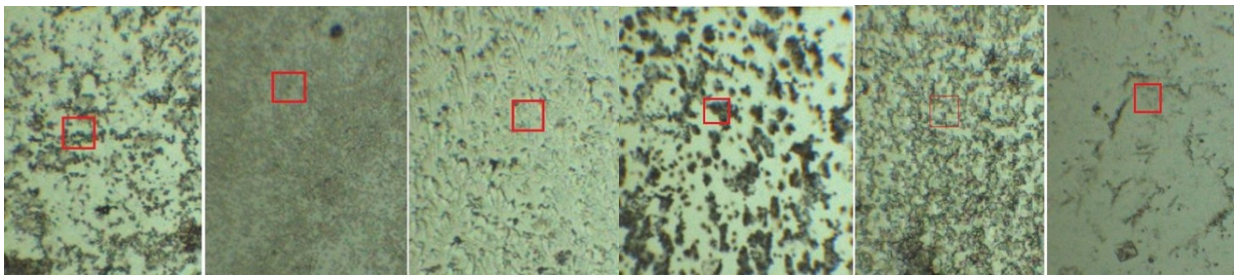

Fig. 4: Group C

Fig. 5: Group D

Males were separated from the females after sperm count and female were observed until delivery of pubs. The average sperm count of the group A is 14.000±1.732 in 1 cm^2, group B is 12±1.000 in 1 cm^2, group C is 23.33±1.528 in 1 $cm^2$and the control group is 11±1.000 in 1 cm^2 (Figures 2-5). Number of pubs was counted for group A, B, C and D (Figure1) and fertility enhancement percentage was calculated as mentioned (table 2).

STATISTICAL ANALYSIS

Results were analyzed by One-Way ANOVA. Software IBM SPSS; Version 17.0.2.

DISCUSSION

Male and female problems are causative factors for infertility. Childless couples suffered by psychological problems which devastasted their marital life. Especially women in our society are much tortured as the period of childlessness increases and sometimes this traumatic condition ends on divorce (Thoma *et al*., 2021). Different medicines are used in assisted reproductive technology (ART) for the treatment of infertility such as ovulation inducing medicines, which leads to complications after childbirth and also increase the risk for other disease diseases. DNA mutation also occurred by modulation in hormonal levels which lead to cancer (Momenimovahed *et al*., 2019). The chromosomal defects were observed in the child born by using *in vitro* fertilization (IVF) and also at high risk to developed cancer in early age (Luke *et al*., 2020).

Herbal medicines are used to treat number of chronic diseases such as diabetes, hypertension, immuno-deficient disorders and cancer in a safer way to improve overall life of patient and protect from deleterious effects of synthetic medication (WHO, 2019). Due to the effectiveness of phytomedicines that are used traditionally to treat abnormalities of reproductive system as well act as anti-fertility agents (Balamurugan *et al*., 2017), the interest in herbal therapy is increased for infertility. These medicines have aphrodisiac potential and treat different male and female disorders with least side effects. *Fagonia arabica* is traditionally used due to various pharmacological activities and also has fertility enhancement effect. Few research studies were conducted to explore the scientific evidence of its clinical use. In this study, Fertility effect of ethanol extract of *Fagonia arabica* was performed to find its potential to treat infertility problems of both male and female. Doses 5mg, 50 mg, 300 mg and 2000 mg/ kg were given and showed no sign of toxicity in acute oral toxicity testing as per OECD guidelines. Determination of effective doses for administered herbal drugs may prevent from the adverse effects (Alahmadi, 2020; Jaradat and Zaid, 2019).

Phytoconstituents mentioned such as tannins, phenol, saponin, alkaloid, terpenes, steroids, glycosides and flavonoids were present in the ethanol extract of the plant. Empirically, traditional medicines were used mainly as a whole extract. The components in the extract of *F. arabica* showed effectiveness for the treatment of infertility which may be result of the combined effect of all its constituents. Secondary metabolites are responsible for the antifertility effect of *F. arabica* extract, such types of compounds as mentioned in table 1 are reported in research studies as effective antifertility agents. For evaluation the fertility enhancement effect, 50 mg/kg Dhamasa Booti (*Fagonia arabica*) was given to both sex of rats in described manner. After mating, sperm counts were noted by observing female vaginal smears under microscope with digital camera and average no of pubs were counted in each group. Average sperm count and number of pubs in test groups compared with control which showed fertility enhancing effect of *Fagonia arabica*. Group C (both male and female rats were treated) showed the highest fertility rate as 87.512%, which revealed it is effective for both male and female. Group A (only male were treated) showed the moderate response as 62.516% fertility rate while Group B (only female were treated) showed the weak response as 37.502% fertility rate.

Oxygen reactive species (ROS) or free radicals are produced on exposure of pollutants, contaminants and during metabolic reactions and which are toxic for cell components. Antioxidants are the group of chemicals which eliminate free radicals and stop their formation. Imbalance between the formation and elimination of free radicals which leads to oxidative stress. Oxidative stress is important biomarker in number of chronic diseases and also cause impairment in proper function of reproduction system such as maturation of oocyte before ovulation and the steps of fertilization and cause endometriosis and other disorders of female reproduction which lead to infertility (Nath and Roy, 2021). Minerals, proteins, vitamins and other micronutrients of *F. arabica* may catalyze different enzymes which neutralize the toxic effects by the reactive species. Antioxidants such as polyphenol, carotenoids, steroids are the constituents present in this herbal extract, which scavenge the reactive species (Tan *et al*., 2018).

Phytomedicines have significant role in treatment of male infertility due to catalyze the enzymes which fight with oxidative stress and improve sperm count and motility. *F. arabica* showed moderate fertility enhancing effect in male rats that's why these are included in dietary supplements to enhance fertility in wild animals which become extinct and cattle to improve different parameters of sperm **(**Abbas *et al*., 2021; Ferramosca *et al*., 2021). Furthermore, those phytomedicines reduce oxidative stress also act as antimicrobial agent. *F. arabica* act as

antimicrobial agent and also used as preservative for semen storage (Gonzales *et al*., 2021; Pini *et al*., 2021).

It is also a requirement that hormonal level must be within normal range to get pregnancy and upto the delivery. Steroidal hormones are synthesized by cholesterol which acts as precursor to improve the hormonal level. Phytochemical analysis showed the extract of *F. arabica* contained steroids just like *Withania somnifera* contains withanolides and lactones and reported as effective in different gynaecological complications as well as provide treatment of infertility (Saleem *et al*., 2020).

The composition of *F. arabica* showed presence of number of constituents. Terpenes and phenols are significant antioxidants which combat the free radicals and serve as antifertility agent. There was an association exist between the intake of antioxidant rich dietary supplements and treatment of infertility. Different isolated compounds such as steroids, triterpenoids, polyphenol (resveratrol, flavonoids) and minerals may also increase the success assisted reproductive techniques (ART). The polyphenols are reported in phytochemical analysis of ethanol extract of *F. arabica*, which may responsible for its antioxidant potential. Flavonoids are the important polyphenol molecules which could be the possible reason of fertility enhancing activity of the plant (Li *et al*., 2019).

CONCLUSION

Fertility testing of *Fagonia arabica* (Dhamasa Booti) has indicated that it possesses highly significant fertility enhancement effect if the test drug is provided to both male and female. Thus, it can be concluded that Dhamasa Booti has pronounced fertility enhancement effect and could lead to a very effective herbal medicine for fertility problems with least adverse effects

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